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AN ANTI-Rh ANTIGEN-ANTIBODY REACTION FACTOR (THE Rh PROTECTIVE FACTOR)

PRELIMINARY REPORT

ALLAN BLOXSOM AND ROSE MATTHAEI

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In a preceding article a report was made on an infant, Baby F. (1), with erythroblastosis fetalis who accidentally received a massive transfusion of Rh-negative blood. Since the course of the hemolytic disease of this newborn infant was so favorably altered by the large transfusion, the possibility that an Rh-negative donor's serum might possess some factor that suppressed or inhibited the Rh antigen-antibody reaction was suggested. If this is true, the serum of Rh-negative donors should be of as much value in transfusions into infants with erythroblastosis fetalis as the Rh-negative red blood cells.

The present study was undertaken to determine, if possible, whether this factor could be demonstrated in Rh-negative blood serums. Accordingly the effects of Rh-negative and Rh-positive serums on the agglutination of Rh-positive cells were studied with commercial Rh-diagnostic serums and with serums containing anti-Rh agglutinins. The commercial testing serum used in this experiment was obtained from the Certified Blood Donor Service in Jamaica, N. Y. The commercial testing serum used in the agglutinations in the included case report was obtained from Dr. J. M. Hill, of the Baylor University Hospital, Dallas, Texas.

Technique for Testing for Rh-Protective Factor

Reagent. Pooled sera (Rh+) of each of the four principal groups, AB, A, B, and O. Pooled cells (Rh+) of each of the four principal groups, AB, A, B, and O. Anti-Rh serum (our preference is the lyophilized serum obtained from the Buchanan Blood Serum and Plasma Center, Dallas, Texas), which is resuspended in 0.9% NaCl solution (normal saline).

Cell suspension. Wash pooled clots of specific type until a heavy suspension of cells is obtained (use saline or Rh+ serum). Centrifuge until cells are loosely packed. If there is any hemolysis, pour off supernatant fluid. However, if cells are so fragile that a second washing is necessary, they cannot be used. Resuspend

cells in type specific Rh+ serum (pooled), making a 2-3% suspension. Use one drop (from a 1 ml. pipette) into each tube of series, both tests and controls.

Blood for testing is obtained by venipuncture and placed in a sterile tube. No anticoagulant is used. Blood is allowed to clot. The clear serum is used for the test. The cells are checked for type and Rh factor.

Technic. A series of ten Kahn size tubes is set up for each serum to be tested and another series of the tubes is set up for each control (a control is set up for each type of blood to be tested; i.e., if there are types A and O in the sera to be tested, a control is set up for each. Like series of tubes are set up to check for iso-agglutinins in each serum to be tested. (In case of donors, the single tube test for iso-agglutinins is satisfactory when there is no history of transfusion or pregnancy.)

For Anti-Rh Antigen-Antibody Reaction Factor

TUBE #	1	2	3	4	5	6	7	8	9	10
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Unknown Rh-serum.....	0.15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Anti-Rh serum.....	0.05									

(Mix and transfer 0.1 cc. to Tube 2. Mix and transfer 0.1 cc. of tube 2 to tube 3, etc., throughout the series, this making the dilutions 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048.)

Cell suspension as described above. Add 1 drop from 1 ml. pipette to each tube.

For Control	1	2	3	4	5	6	7	8	9	10
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Rh + Serum (group specific).....	0.15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Anti-Rh Serum 0.05 cc. (Mix and transfer as above, to successive tubes.) Cell suspension as described above. Add 1 drop from 1 ml. pipette to each tube.

Test for iso-agglutinins. Set up series of tubes as for the control, using type specific pooled serum but use 0.05 cc. of serum to be tested IN PLACE OF the commercial Anti-Rh serum in the first tube. Proceed as in other series of tests.

Allow to stand at room temperature overnight OR incubate in 37° waterbath for 45 minutes. If room temperature drops as much as 15° below 37°, during the over night incubation period, place racks of tubes in waterbath for 15 minutes before centrifuging.

Centrifuge tubes at approximately 2500 rpm for 4-5 minutes. Read promptly. Tilt tube gently (any rough shaking of tube will completely mask a ± reaction). Read before a frosted light (Kahn or microscope lights are satisfactory). Readings are + (large, distinct aggregations of cells, with no outflow of single cells); ± (smaller aggregations of cells, with some dispersion of cells), - (no aggregation of

cells). If reading is a weak \pm , reading may be facilitated by noting aggregate at bottom of the tube. \pm shows a rough, irregular edged circle, while $-$ shows a smooth circle with single cells flowing from the sides.

STUDY 1

In order to provide controls for the various phases of this problem, the titer of the commercial Rh-diagnostic serum was determined on Rh-positive cells of stock group O and stock group A blood as well as on the blood of Baby F. (group A), dilutions being made with isotonic solution of sodium chloride. The results of the control studies are shown in table 1.

The commercial anti-Rh testing serum showed titers of 1:64 with the three groups of Rh-positive cells used in the following studies.

TABLE 1
Titer of Commercial Testing Anti-Rh Serum

	DILUTIONS OF ANTI-Rh TESTING SERUM							
	1:4	1:8	1:12	1:16	1:24	1:32	1:48	1:64
Testing with red blood cells:								
Rh-positive (O).....	+	+	+	+	+	+	\pm	\pm
Rh-positive (A).....	+	+	+	+	+	+	\pm	\pm
Rh-positive (A) (Baby F) ..	+	+	+	+	+	+	+	+

(All dilutions were made with isotonic solution of sodium chloride.)

STUDY 2

This study was done on the serum of the donor, Mr. B., who supplied the blood for the massive transfusion previously reported. It was done to determine, if possible, the existence of any anti-Rh antigen-antibody reaction factor in his blood. The results are shown in table 2.

When the commercial anti-Rh testing serum was variously diluted with the serum of Mr. B., there was evidence of anti-Rh antigen-antibody reaction factor activity in agglutinations of and above 1:10 but complete inhibition of agglutination in dilutions of and above 1:40. When isotonic solution of sodium chloride was used as the diluent, agglutination occurred in dilutions of 1:64.

STUDY 3

This study consisted of examining the effects of various Rh serums from Rh-positive and Rh-negative donors on the red blood cells of

TABLE 2

Anti-Rh Antigen-Antibody Reaction Factor Titer of Serum of Donor Mr. B. for Infant F.

	DILUTIONS OF ANTI-Rh TESTING SERUM						
	1:5	1:10	1:15	1:20	1:30	1:40	1:60
Testing with red blood cells of infant F. Rh-positive (A).....	+	±	±	±	±	-	-
(All dilutions were made with serum of donor Mr. B. for infant F.)							
Control shown in table 1.....	+	+	+	+	+	+	+

TABLE 3

Effect of Incubation of Various Rh-Negative and Rh-Positive Blood Serums on the Rh-Positive Cells of an Infant with Erythroblastosis Fetalis

	DILUTIONS OF ANTI-Rh TESTING SERUM						
	1:5	1:10	1:15	1:20	1:30	1:40	1:60
Testing with Rh-positive (A) cells of infant F. Using serum of Rh-negative donor:							
Mrs. P.....	+	±	±	±	-	-	-
Mrs. C.....	+	+	±	±	-	-	-
Mr. B.....	+	±	±	±	±	-	-
Using serum of Rh-positive donor:							
"x".....	+	+	+	-	-	-	-
"y".....	+	+	+	+	+	+	+
"z".....	+	+	+	+	±	±	±

(All dilutions were made with serum from Rh-negative and Rh-positive donors.)

Baby F., who received the massive transfusion. The results are shown in table 3.

The most striking result apparent from table 3 is the fact that Rh-negative blood serums were much more effective in inhibiting agglutination than Rh-positive blood serums. The red blood cells from

Baby F. used in this study sixty days after the massive transfusion probably contain some Rh-negative red blood cells. The serum of the donor Mr. B., who gave the massive transfusion, is shown to be effective in preventing agglutination of the infant's cells by potent commercial anti-Rh testing serum. This fact, of course, suggests the

TABLE 4

Effect of Incubation of Various Rh-Negative and Rh-Positive Blood Serums on Rh-Positive Cells Tested with Commercial Rh Testing Serum

	DILUTIONS OF ANTI-Rh TESTING SERUM						
	1:5	1:10	1:15	1:20	1:30	1:40	1:60
Testing with							
Rh-positive (A) red blood cells using serum of Rh-negative (A) donors:							
Mrs. P.....	+	±	±	-	-	-	-
Mrs. C.....	±	±	-	-	-	-	-
Mr. B.....	-	-	-	-	-	-	-
Rh-positive (O) red blood cells using serum of Rh-negative (O) donors:							
Mrs. H.....	±	±	±	±	±	±	-
Mr. McD.....	-	-	-	-	-	-	-
Rh-positive (A) red blood cells using serum of Rh-positive (A) donors:							
"x".....	±	±	±	±	±	±	±
"y".....	+	+	+	+	+	+	+
"z".....	+	+	+	+	+	+	±
Rh-positive (O) red blood cells using serum of Rh-positive (O) donors:							
"a".....	±	±	±	±	-	-	-
"b".....	±	±	±	±	±	±	±

(All dilutions were made with serums of Rh-negative and Rh-positive donors.)

reason for the beneficial results on erythroblastosis fetalis when Rh-negative blood is used for transfusions. It further suggests that there may be a beneficial result or effect on the fetus exposed to anti-Rh agglutinin if that mother during gestation is given serum from Rh-negative donors at regular intervals.

STUDY 4

The effect of Rh-negative and Rh-positive blood serums on agglutinations of Rh-positive cells with the commercial anti-Rh testing serum was studied. The results are shown in table 4.

There is a rather noticeable difference between the degree of inhibition of agglutination with the use of serums from Rh-negative donors and the inhibition produced by Rh-positive serums. One Rh-positive serum ("a") acted somewhat like Rh-negative serum and there is some hereditary evidence that this donor is heterozygous instead of homozygous. A further study is being done on persons whose blood reacts in this fashion, and it may be possible to predict whether an Rh-positive person is heterozygous or homozygous from the presence or absence of the anti-Rh antigen-antibody reaction factor in his serum, as true Rh-positive donors seem to have little or none of this factor in their serums. From table 4 it is evident that the Rh-negative male donors tested, not having received a transfusion, show considerable anti-Rh antigen-antibody reaction factor in their serum.

COMMENT

Rh-negative blood serums, particularly those of males that have been tested, contain an anti-Rh antigen-antibody reaction factor that inhibits the Rh antigen-antibody reaction. The presence of such a factor can furnish an answer to a number of questions about hemolytic disease of the newborn (erythroblastosis fetalis). Such a factor, as will be pointed out later, could be of great value in the treatment and possibly in prevention of this condition.

Levine expressed the opinion that one should expect erythroblastosis fetalis to be a disease exclusively of the fetus, with spontaneous cure at birth. The presence of such an inhibiting Rh antigen-antibody reaction factor circulating in Rh-negative blood and holding this reaction in abeyance or to a minimum until the fetus is removed from its sphere of influence would explain the mechanism in many instances of rapid development of erythroblastosis fetalis shortly after birth.

As has frequently been observed, the titer of the maternal anti-Rh agglutinins is not necessarily proportionate to the severity of the fetal disease. The reason for this lack of correlation between the severity

of the disease in the fetus and the concentration of agglutinins in the mother's blood is stated by Potter to be unknown. The presence of an anti-Rh antigen-antibody reaction factor can explain this phenomenon. Women whose infants are affected with the most severe form of the disease and in whom it is impossible to demonstrate agglutinins may have little or no inhibiting anti-Rh antigen-antibody reaction factor present, so that the infant receives no protection from his mother. In mothers having a high titer and a high neutralizing anti-Rh antigen-antibody reaction factor, the infant may be given considerable protection and be only mildly, if at all, affected.

The presence of this anti-Rh antigen-antibody reaction factor in Rh-negative blood serum and possibly to some extent in heterozygous Rh-positive blood serum further explains why Rh-negative blood is much more effective in transfusions for infants with erythroblastosis fetalis.

Clinical experimental work with mothers given serum from Rh-negative male donors during gestation of expected erythroblastotic infants in an effort to inhibit or minimize the injury to their fetuses will be reported. At the present time the blood of an Rh-negative mother having a normal infant after a previous infant who died of erythroblastosis fetalis has been studied. Anti-Rh agglutinins that were present days before delivery in this mother's blood disappeared twelve hours after delivery of the normal infant. The anti-Rh antigen-antibody reaction factor increased in this mother's blood serum approximately fourfold. Anti-Rh agglutinins were demonstrated also in the normal infant's cord blood, and the anti-Rh antigen-antibody reaction factor was present in the same titer as in the mother's blood serum.

A question may arise as to whether or not the prevention of agglutination of Rh-positive cells in the presence of a potent anti-Rh agglutinin serum is the result of the "blocking anti-bodies" described by Wiener. These "blocking antibodies," or univalent agglutinins, were independently discovered by Race and by Wiener. The "blocking antibodies" combine with their antigen, but the reaction stops at this point in vitro and no agglutination or hemolysis occurs. The presence of these univalent agglutinins can be demonstrated by adding potent anti-Rh-agglutinating serum to a mixture of patient's serum that has

been incubated with group O, Rh-positive cells. If the patient's serum does not contain any "blocking antibodies," strong clumping should now be evident. Should the patient's serum contain "blocking antibodies," the Rh-positive red cells will remain unagglutinated, or the clumping will be weakened. The anti-Rh antigen-antibody reaction factor is different from the "blocking antibodies" because it occurs in the serums of Rh-negative male donors who have received no blood transfusions and obviously could not be sensitized by pregnancy. Second, ten infants with erythroblastosis fetalis have received large transfusions to date from selected Rh-negative male donors with the higher titers of the Rh protective factor for the effect of this factor. The response has been excellent, and all infants have done well, something one would not expect if this protective factor is a "blocking antibody."

The paralleling clinical and laboratory evidence showing the anti-Rh antigen-antibody reaction factor to be a natural protective factor present in the blood of Rh-negative persons is being published. It is suggested, therefore, that this natural Rh-reaction inhibitory factor be called the Rh-protective factor.

The serums of Rh-positive men who from their offspring are known to be heterozygous will be examined for evidence of the anti-Rh antigen-antibody reaction factor. If this is consistently found, it may constitute a simple determining test for the Rh heterozygote.

SUMMARY

1. The serums of the Rh-negative bloods used in the studies of this article have been shown to contain an anti-Rh antigen-antibody reaction factor that inhibits the Rh antigen-antibody reaction.

2. The serums of the Rh-positive bloods used in the studies of this article have been shown to contain no anti-Rh antigen-antibody reaction factor except for the small amount found in one man who is heterozygous.

3. At the present time it is believed that this anti-Rh antigen-antibody reaction factor is a specific inhibiting substance for the Rh reaction. The natural occurrence of this anti-Rh antigen-antibody reaction factor serves as a vital protective means for Rh-negative persons and for infants subject to hemolytic disease of the newborn.

Serum from Rh-positive persons in the few instances tested nullified the inhibiting power of the serum from Rh-negative male donors.

4. The value of both cells and serums of Rh-negative bloods, with, probably, the serums being of greater value for transfusions in infants having erythroblastosis fetalis due to anti-Rh agglutinins, is believed in at present.

5. The males tested in this study showed in their blood serums greater amounts of this anti-Rh antigen-antibody reaction factor, and it is possible that blood used from such donors will be more effective in the treatment of hemolytic disease of the newborn (erythroblastosis fetalis).

6. The prevention of hemolytic disease of the newborn (erythroblastosis fetalis) is an obstetric problem. It is hoped that the use of suitable Rh-negative blood and blood serums at regular intervals before delivery in the light of its inhibitory effect on anti-Rh agglutination in vivo and in vitro, as demonstrated in this article, may result in the salvage of a larger number of those infants.

7. The presence of the anti-Rh antigen-antibody reaction factor in the serums of Rh-positive males may point to the fact that those males are heterozygous.

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PSYCHOTHERAPY IN GENERAL MEDICAL PRACTICE

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Illnesses of emotional origin—the neuroses and allied disorders—constitute a large part of the ill health for which people seek medical help. Estimates vary, ranging from the neighborhood of one-third of all patients up to two-thirds of all patients, as the proportion of those in whom the most significant cause of ill-health is of emotional or neurotic character. The larger estimates include many who do have some organic pathology which is not considered an adequate explanation for the complaints presented. On the more conservative estimate, about one patient in every three entering a doctor's office needs psychotherapeutic assistance; yet medical education has not prepared physicians well to meet this need, or even in fact to recognize properly the neurotic illnesses at the stages in which they respond best to psychotherapeutic treatment. An attempt is made herein to outline a perspective on the therapeutic possibilities and principles by which the physician may be aided in his treatment of the neuroses and allied forms of illness.

Let us plunge at once into the consideration of a type of neurotic illness familiar to all physicians—the anxiety reaction, or the “jitters.” The anxiety reaction may proclaim itself in obvious clinical manifestations of tension, tremulousness, restlessness, moist palms, rapid or irregular action of the heart, and “butterflies” in the stomach, or may be masked behind a patient's complaint of “heart trouble”, “stomach trouble” or “thyroid trouble”, or it may be half concealed by some once-respectable medical term like neurocirculatory asthenia or shell-shock.

Let us for our purpose suppose that the diagnostic problem is settled—the patient is found to have a neurotic anxiety reaction. The problem is then therapeutic, and this therapeutic problem is in the setting of general medical practice. What help can be given such a patient?

Well, in the general run of medical practice there will be a certain

proportion of patients, a rather small proportion, who spontaneously respond very well when informed that their symptoms are of emotional origin. Such a patient may be aware of a persistent source of uncertainty or worry which can be decisively settled and whose settlement relieves his anxiety.

More commonly, however, the patient is not able without help to understand the personal meaning of his anxiety. He is therefore unable to take decisive action, and so continues in the anxiety state, or he may even get more anxious over the added worry that something is going wrong in his mind about which he can do nothing. Is this a problem about which the general practitioner can do some useful therapy, or is it necessary to call in a psychiatrist, and, if so, where are we to get the tens of thousands of psychiatrists needed? These are pressing problems which come up at once when one faces the issue. Of course you know and I know that the issue is usually not faced. The doctor finds some "suggestive evidence" of possible avitaminosis or endocrinopathy. He embarks upon a series of therapeutic trials, trying to bolster up his own hope, against his better clinical judgment, that this capsule or that injection will somehow do the therapeutic trick. It usually doesn't. This is sometimes known as giving the patient the benefit of the doubt, but that is not the correct way to state it. Unfortunately, when the first flush of suggestive benefit has faded, the patient has usually added another layer of psychopathology to the anxiety reaction,—he has become in some measure hypochondriacal or neurasthenic, confirmed, so to speak, in the pill-taking faith, or goes on to seek surgical intervention, so the still remaining psychotherapeutic task is multiplied in complexity and difficulty.

Let us return then to the question, "Can the general practitioner do some useful psychotherapy in those anxiety reactions which do not promptly cure themselves when reassured as to organic integrity?" Opinions differ on this question. Everyone must admit that there do exist certain rare physicians with remarkable powers of intuitive insight and personal charm who are able to give much support to anxiety-ridden patients. The practical question concerns, however, the more nearly average physician,—the one without this "magic touch." Can he learn to give useful psychotherapy to patients in anxiety states?

My own opinion is, "Yes, but with two important qualifications." First, one has to give some serious study and effort to mastering the intelligible principles of psychotherapy and, second, one needs to establish serious consultative contacts with some other physician or physicians likewise seriously interested in psychotherapy. You will note, and perhaps be surprised at my setting forth the necessity for consultative contacts with another physician. You would perhaps be less surprised if I specified consultative contacts with a well-trained psychiatrist, which would of course be fine. But another general practitioner will do. I wish to return to this consideration later.

The first necessary step for the general practitioner in doing useful psychotherapy is the mastery of some intelligible principles of psychotherapy. This involves a scientific and professional understanding of human nature which has seldom in the past been presented to the physician at the right time, namely, in medical school. The medical schools are, one by one, in varying degree, moving toward a better fulfillment of this obligation. The physicians of the future should be much more adequately informed about personality functions and personality disorders than the physicians of our generation. But it is my obligation to try to say now what constitutes the minimal grasp of principles which the present general practitioner would need to know to undertake psychotherapy.

I do not believe that doctors should attempt to do psychotherapy blindfolded—that is to say, in ignorance of psychopathology and psychodynamics. In considering the treatment of neurotic anxiety reactions, one should have some fairly clear concept as to the nature of this disorder. Like other neurotic illnesses, the anxiety reaction is a symptom of unfinished business,—some internal conflict or issue which the patient has not been able to settle, and which therefore haunts him. I would go further and express my agreement in general with those who say that neurotic anxiety reactions are symptoms of difficulty in managing some inner impulses. This is usually unknown to the patient. The patient commonly believes his anxiety is aroused by circumstances, but that belief just misses the bull's eye. The circumstances arouse certain unacceptable impulses, and the patient's difficulty in handling those impulses is what stirs up the anxiety. I express myself rather dogmatically on this point because my observations and

experiences have convinced me of the general validity of this conception of the anxiety reaction, and I consider it of great strategic importance for therapy. It is not always necessary for success in psychotherapy to know the original cause of the patient's conflict of impulses, but it is a great help to know something of its nature in the individual case, that is to recognize what sort of impulse is aroused by the circumstances and to mobilize the patient's resources for dealing decisively with that sort of impulse.

Not only is the neurotic patient ordinarily ignorant of the nature of the impulses concerned in his anxiety reaction; he doesn't want to know, if he can avoid it, for these impulses are usually of a sort that would shame him. It is just at this point that the emotional nature of the patient-physician relationship has its crucial importance in psychotherapy. People can sometimes endure to take off their concealments and look at themselves in the doctor's presence, in a way they can do in few other situations. If the doctor can stand the view, the patient can too. Some doctors can't stand it,—they are made very uncomfortable by raw human nature. Such a doctor should not try to do psychotherapy. Human nature is not all pretty on the inside. There is a good deal of meanness and low animal nature native to everyone. In recent decades those recesses of human nature concerned with sex have been widely publicized. There is some reason to believe that people in general now have a less sordid view of sex than prevailed a generation ago. Nevertheless, the physician who undertakes to do psychotherapy should be prepared to make the acquaintance of some fairly sordid aspects of human nature, sexual or otherwise. Those who have to steel themselves to endure the sordid revelations of human impulses do not do very good psychotherapy. It seems to require a fairly robust faith in the human race,—a faith which may grow with a doctor's experience, as his knowledge of his patients' lives broadens and deepens. One of the basic principles of psychotherapy is to permit the patient to be straight-forwardly honest about himself. I say "permit" advisedly because I find that physicians often automatically shut off the patient from telling the candid truth because of the overprotective medical tradition.

The parallel to this principle of straightforward candor is for the physician to display interest in, and appreciation for, the patient's

assets and good points. In every medical history designed for psychotherapeutic purposes there should be a section which tells of the patient's best periods and the pattern of the patient's life at those periods. This provides concrete information about the patient's level of personality functioning at its best, but, more significantly, the detailed conversation with the physician about the periods of good functioning may revive afresh some of the constructive and positive personal attitudes which made one function well at that time.

In reading some of the modern psychiatric literature and especially in attending psychiatric movies, the American public has been fascinated by the search for that one childhood incident which was *the cause* for today's neurosis. Well, a great many patients are helped to make very good recoveries without anyone's ever learning *the cause* in this sense. Indeed, it may generally be doubted whether it is proper in most cases, to speak of *the cause* as a single incident, since the crux of a neurosis often lies in a conflict of attitudes or a failure in personality development, which had a long and intricate development without any one specific incident as the cause. What is of much greater importance than to find the cause is to discern the meaning of the symptoms, the motivation, in terms of the issue at stake in the life situation to which the neurosis is the patient's reaction. There are no wholly dependable tricks of the trade which can be relied upon to reveal this meaning when the patient is reluctant or unable to talk. It happens to be true that mute patients—those who do not talk—happen to be a special personal interest of mine, and I have developed certain skills in establishing communication with them, but those skills are not the business of the general practitioner. I do, however, wish to offer one suggestion of considerable value in trying to figure out the meaning to the patient of a life-situation which seems to the physician trivial and inappropriate as the occasion for so much anxiety. That suggestion is to evaluate the level of the patient's emotional maturity, which may be done, approximately, by a suitable interview and history. I do not mean merely to decide that the patient is immature. That is somewhat too general. What I mean is to locate approximately the patient's position on a scale of emotional growth, the principal positions being denoted by the terms infantile, childish, early adolescent, late adolescent and adult. These terms, as I use them, summarize

the level to which the patient's personality has grown in its emotional maturation, rather specifically in regard to dependence and responsibility. For teaching purposes I have defined these terms in the following brief fashion:

Infant level: complacently or petulantly dependent.

Child level: making excuses for self, but expecting perfection in others.

Early Adolescent level: "Independent" of authority but much concerned about contemporaries—badges, cliques, prestige—"showing off"; strenuously adventurous.

Late Adolescent level: Very self-consciously idealistic, or cynical or romantic; sophisticated or sophomoric "line"; radical or extremist in belief but less adventurous in action.

Adult level: More absorbed in work or family than in self-assertion; reconciled to limitations through satisfaction in real achievements; accepting and sharing responsibility without "fuss."

A biographical review and interview which provides the evidence for evaluating the patient's level of emotional maturity generally serves also to reveal attitudes involved in the patient's neurotic reaction to the current life-situation, giving the doctor a fairly clear idea of the emotional meaning of the situation to the patient, even though the patient is still in the dark. Just how well the general practitioner can learn to size up patients' attitudes and the levels of emotional maturity must of course vary with one's insight and experience, and the amount of study and thought given to the matter. It is not wholly a gift. It is a skill that can be developed by exercise.

Granted then that the physician has a certain degree of skill in evaluating people's attitudes in relation to life-situations and the level of maturity, and is thereby equipped to discern the meaning of the patient's neurotic anxiety reaction to a situation seemingly inappropriate, such a physician is, in a fair measure, prepared to undertake psychotherapy, and that psychotherapy will consist largely in the thoughtful and respectful consideration with the patient of how the situation might be met more effectively, not by an ideal person, but by the person who is the patient, using to the best advantage the assets and attitudes which he has shown in periods of good adjustment. When I say consider with the patient, I mean consider, I do not mean

to urge or to exhort, but to consider things together with mutual respect. The doctor need not be excessively humble or passive about this, for in his ordinary quiet and objective way the physician often exerts tremendous personal influence which can be brought to the support of the patient's weakened self-confidence. This personal influence in the patient-physician relationship is the real moving force in psychotherapy. The whole art of psychotherapy depends largely on learning how to exert this special personal influence strategically to the patient's best advantage in finding a better way to meet life-situations. That is why it is important to know something about patients as persons and about the dynamics of neurotic reactions.

This brings me to the second qualification which I specified when I said that the general practitioner could do effective psychotherapy, with two qualifications. The first I have just finished discussing in a general way,—the mastery of a certain knowledge of human nature and the psychodynamics of neuroses. The second qualification was that he should establish serious consultative contacts with some other physician or physicians likewise interested in psychotherapy; and I would like now to consider with you the meaning and purpose of this point. The most active agency in psychotherapy is the emotional relationship and interaction between patient and physician, and I seriously doubt if this rather intensive personal relationship can be employed effectively and yet with professional discretion and judgment unless the physician attempting to do so has a trusted professional colleague to whom he can make objective reports and thereby keep himself objectively oriented.

There has been much discussion in recent years of group psychotherapy. What I am attempting to say now is the converse of ordinary group psychotherapy—namely, that the psychotherapist should be a member of a group numbering at least two who share a common and serious interest in following each other's psychotherapy. They may in this fashion maintain each other's objectivity in the employment of a rather subtle kind of subjective influence—the emotional relationship between patient and physician.

Let me tell you some of the reasons which lead me to emphasize this point. It has been my good fortune to become acquainted in several sections of this country, and in the Army, with a number of

physicians who have become individually interested in psychotherapy and have attempted individualistically to do psychotherapy. The physician attempting to do psychotherapy all on his own, so to speak, is not usually aware of the emotional intensity of the patient's reactions to him until it suddenly erupts explosively, whereupon he over-reacts in a defensive or aggressive way and spoils the whole therapy; or perhaps the doctor himself gets emotionally involved in a way which is not helpful. After a few experiences of these kinds the interested physician usually adopts one or the other of two opposite patterns. Either he becomes so coldly professional and impersonal in attitude as to lose most of the potentiality for effective psychotherapy, or he drifts into a series of intensive personal relationships with patients which cease to have therapeutic value to the patients and which become in effect a special neurosis of the physician. To avoid these two extremes of reaction, nothing is so helpful as the habit of objective report and consultation with another professional associate who has a similar sympathetic but practical interest in psychotherapy.

It is also helpful to point out another reason for consultative contacts to maintain objectivity. The doctor who is unsuccessful in the attempt to exercise his personal influence in psychotherapy suffers a more disturbing personal frustration than when his prescriptions are unsuccessful. This experience is hard to take all alone, but if two or more share each other's experiences in an objective way each knows that the others are also frustrated at times, and consequently does not take his own defeats so much to heart.

We have been considering, in some detail, the considerations which seem to me important and necessary for the physician in general practice to do useful psychotherapy for patients who are in the condition called anxiety reaction.

There is a large group of neurotic illnesses which might be comprehended under the headings of tension states and fatigue states. By and large these are simply cases of anxiety reaction in which the effects of anxiety have come to loom larger than the anxiety itself. The principles of treatment are essentially the same,—to arrive at a sound personal diagnostic formulation through mutually respectful consideration with the patient of his reaction and the life-situation, and to utilize the personal influence of the patient-physician relationship to

provide a corrective and supportive emotional experience, whereby the patient may come to deal with his situation more effectively and maturely.

There is, however, a more complicated level of neurotic illness, which may be more frustrating to one's psychotherapeutic ambitions. I refer now to the neurotic defense mechanisms, such as hysterical conversion, hypochondriasis, circumscribed phobias, and obsessive-compulsive adjustments. Expressed in the broadest terms, these neurotic defense mechanisms may be considered as devices for evading major anxiety reactions by unconscious mechanisms which gain one special sympathy or excuse one from facing intolerable situations. Some of these conditions are very difficult to treat psychotherapeutically, others respond well and promptly. It is not always possible to tell which will respond well. Success in their psychotherapeutic treatment is made more difficult, in general, by the passage of time, by the multiplication of operations and special tests, and by the taking of medicines. In general, the psychotherapy of the neurotic patient with well-established neurotic defense mechanisms is a task for the specialist rather than the general practitioner. One may hope, however, as psychiatric training improves in medical schools, that more and more medical men, even though not specialists in psychiatry, will be able to treat such patients successfully.

There is one special group which I wish to discuss because of its prevalence and general medical importance. I refer now to the group of patients called by Alvarez the "constitutional inadequate." These are persons who are always tired or suffering various aches and pains because they try to live at a level which is beyond their physiological means. In this sense the concept of constitutional inadequacy has a certain validity. In the main I consider it more accurate to pin down at least a large proportion of these cases under the designation of obsessive perfectionists. In other words it has seemed to me that such patients have not been able to be reasonably lenient on themselves because they are driven by neurotically obsessive attitudes. They tend therefore to alternate between a worn-out neurasthenic state in which they are dreadfully tense, irritable and fatigued and have to be inactive, and a state of obsessive overwork which drives them again into the fatigue state. Some of these patients can be saved from what

is a rather miserable pattern of life by psychotherapy directed at the relief of the obsessive mechanism. The general practitioner sees great numbers of these patients, and may be able to help a few by psychotherapy of a fairly blunt and straightforward type designed to relieve the obsessiveness.

In concluding this discussion of psychotherapy in general medical practice, I will summarize the main points by saying that many patients showing anxiety reactions may be aided considerably by psychotherapy which can be done by the general practitioner who is willing to put in a certain amount of hard work to gain an understanding of the matter and who will maintain consultative contacts with a colleague likewise seriously interested. Many patients in tension states can likewise be aided. The prospects are not so good for the psychotherapy of well established neurotic defense reactions by the general practitioner, but it does seem potentially useful for him to attempt psychotherapy on a very numerous group of patients who are neurotically driven by obsessive perfectionism to exertions beyond their physiological capacity to sustain.

The development of a moderately successful skill in psychotherapy should be a considerable source of gratification to the practitioner in providing a feeling of decent competence for dealing with that one-third or more of his patients whose primary medical need is for psychotherapy. The insight into human nature, which is a by-product of psychotherapeutic success, enriches one's professional life through a very interesting and gratifying appreciation of the constructive potentialities of human beings.

THE RESISTANCE OF THE YOUNG RABBIT TO THE DIABETOGENIC EFFECT OF ALLOXAN

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INTRODUCTION

Following the discovery by Dunn, Sheehan, and McLetchie (8) that the ureide of mesoxalic acid, alloxan, injected into the rabbit in doses of 300 mg. per kg. could be depended on to produce an extensive lesion of the islets of the pancreas with characteristic symptoms, many reports concerning alloxan diabetes in the laboratory animal appeared in the literature (1, 2, 3, 4, 7, 13, 19).

Those investigators have dealt with the production of alloxan diabetes in the adult animal, and, so far as we have been able to determine, no studies of the susceptibility of very young animals to alloxan have been heretofore carried out. Friedgood and Miller (10) found that the fetal rat is not susceptible to alloxan. The question arises, therefore, at what period of life does the animal become susceptible to alloxan?

METHOD

The young rabbits in our experiments were obtained from adults of mixed stock bred in the laboratory. In the adult rabbit it has been demonstrated that a 200 mg. per kg. dosage of alloxan produces a permanent diabetic state (1, 6). In this series, a dosage of 300 mg. per kg. was employed before the thirtieth day after birth; on the thirtieth day and thereafter a dose of 200 mg. per kg. was employed. Thus, the young animals were given a dosage 50% greater than that which produces diabetes in the adult.

No animal was starved prior to injection. Intracardiac injections of alloxan were performed with a tuberculin syringe. Although the injection was rapid, blood was drawn into the syringe at the beginning, during the course of, and at the end of injection in order to confirm the intracardiac route. (Leech and Bailey have found that alloxan disappears almost completely from the blood within two minutes (21).)

The diabetic state of the animal was determined by the blood sugar level, the blood being withdrawn from the animal by intracardiac puncture. This procedure was employed until the veins of the ear were of adequate size. The Folin-Malmros microtechnique (9) was employed to determine the blood sugar values. In this series, animals with blood sugar levels exceeding 300 mg. per 100 ml. were considered to be diabetic (13). In no instance were the animals intentionally starved prior to a blood sugar determination. The frequent and prolonged absence from the mother which this procedure would entail, and the delicate nutritional status of the newborn rabbit, rendered fasting impracticable. From the majority of litters employed in this experiment one or two animals were selected as controls, blood sugar values being determined concurrently with those of the injected animals. These blood sugar values served as normal values and as controls over the blood sugar technique.

RESULTS

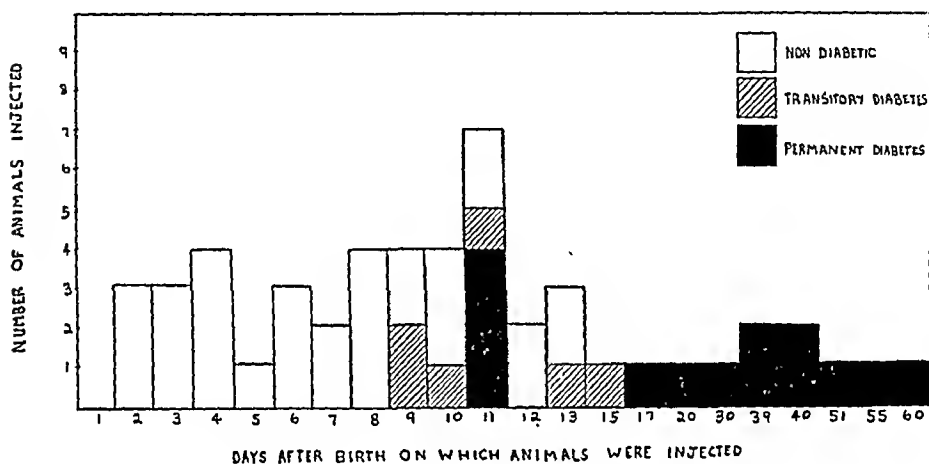
Fifty-one animals varying in age from two through sixty days after birth were injected with alloxan. Thereafter, the animals were weighed and blood sugar determinations were made at intervals of a few days over periods ranging from two weeks to eight months (see Table IA). Weights and blood sugar levels of ten litter mate controls are to be found in Table IB.

The distribution of diabetic animals in the total number injected is shown in Graph I. From this graph it will be noted that in none of the twenty animals injected prior to the ninth day were we able to demonstrate a diabetic state. Of the twenty-one animals injected from the ninth through the fifteenth day, four developed a permanent diabetes, six developed a transitory diabetes, and eleven failed to become diabetic. All of the ten animals injected after the sixteenth day of life developed a permanent diabetes.

This transitory alloxan diabetes in the laboratory animal, which we noted in six instances, has been reported in the literature (5, 6, 19, 21). We have classified an animal as a transitory diabetic provided that at least once following injection he is hyperglycemic to the extent of 300 mg. per 100 ml. We consider an animal to have a permanent

diabetes if the hyperglycemia is maintained. One animal (76-A3) has remained hyperglycemic over a period of one year. The remainder were either sacrificed or died during the course of their diabetes.

Statistical analysis of these data indicates the significance of the failure to produce demonstrable diabetes in the twenty animals injected prior to the ninth day. The expected incidence of diabetes in these animals would be 64.5%, this being the incidence of diabetes in the group injected on the ninth day and following. The standard error of the difference between the two groups is 8.6. The experimental



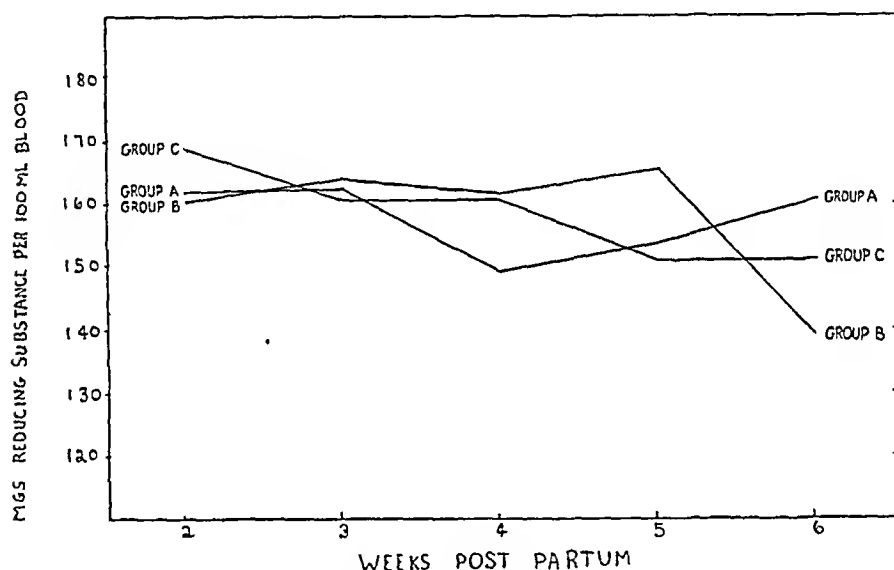
GRAPH I. Age distribution of animals injected with alloxan per day post partum and the incidence of diabetes.

incidence in the younger group was 0.0%, or exactly 7.5 standard errors from the incidence in the older group, 64.5%.

The blood sugar determinations were compiled and studied in the following manner. First, it was decided to determine the blood sugar levels in the normal, non-fasting, nursing animal. Blood sugar values were determined on each of twenty-eight animals several times every week from the second through the sixth week after birth. The values for all animals during each week were grouped, and an average value computed (Group A). The non-diabetic injected animals in the series were divided into two groups. Group B comprises all animals injected prior to the ninth day after birth. Group C includes those animals injected on the ninth day or after which failed to develop a

demonstrable diabetes. An average weekly value for each group was calculated by the method employed for Group A. The average blood sugar levels of each separate group are compared in Graph II. Statistical studies indicate that the blood sugar levels of Groups B and C do not deviate significantly from the blood sugar levels of Group A during corresponding weekly intervals.

In Table IA it will be noted that several relatively high blood sugar values were found in animals which have not been considered diabetic. When these values were obtained, control litter mates had similar



GRAPH II. Average weekly blood sugar levels of nondiabetic animals.

blood sugar levels (see Table IB). It was noted in general that the average blood sugar levels of the young, nursing animals were higher than the average blood sugar levels of the adult animal.

Duffy (6) noted that the appearance of diabetes was correlated with a loss of weight. This is indicated in our series: 76-A3, 98-1 (reinjection), 98-2 (reinjection), 105-1, 105-2, 105-3, and 105-A9 showed a definite loss of weight at various periods after injection; 57-1, 76-A2, and 71-A2 did not show this relationship; while the correlation in 75-1 and 76-A5 is questionable. This relationship between weight and the diabetic state is demonstrated in Table IA.

One observes in Table IA that the period required for the development of a demonstrable diabetes varies greatly. On the first blood sugar determination following injection thirteen animals were diabetic within the first three days. This includes one animal which died in hypoglycemic convulsions. One animal was found diabetic on the fifth day, the first analysis following injection. Four other animals were found diabetic between the seventh and twelfth days following injection. Another animal was found to be diabetic on the fifteenth day after injection. In one animal, 76-A3, we are unable to determine the time of the appearance of the diabetic state. During the first two weeks after injection diabetes could not be demonstrated. Due to the vacation period no analyses could be made on this animal until five months after injection. At this time the animal was found to be diabetic. A number of investigators (2, 11) has reported that typical diabetes mellitus in the adult animal will develop during a short interval (24-36 hours) after injection of a diabetogenic dose of alloxan. However, Leech and Bailey (21) have indicated that the diabetic state may not appear until the first or second week following injection.

The question arose as to whether alloxan diabetes could be produced at a later period in those animals which had failed to develop diabetes when initially injected in the neonatal period. For this purpose four of those animals were chosen: 60-1 was injected with 200 mg. per kg. of alloxan two hundred days after the initial injection of 300 mg. per kg. on the sixth day after birth. The pre-injection blood sugar level was 122 mg. per 100 ml. of blood. A blood sugar level of 59 mg. per 100 ml. was obtained sixteen hours after the injection. Twenty-two hours after injection the animal died in hypoglycemic convulsions. 98-1 and 98-2 were injected with 200 mg. per kg. of alloxan thirty-one days after the original injection of 300 mg. per kg. of alloxan on the eighth day after birth. The pre-injection blood sugar level of 98-1 was 147; and that of 98-2 was 105 mg. per 100 ml. On the day following injection blood sugar levels of over 400 mg. per 100 ml. were obtained in both animals. And on three other occasions during the week following injection the blood sugar levels exceeded 400 mg. per 100 ml. These animals were maintained on insulin for eight days, and then sacrificed. 101-3 was injected with 200 mg. per kg. of alloxan fifty-one days following the initial injection of 300 mg. per kg. on the

ninth day after birth. The pre-injection blood sugar level was 109 mg. per 100 ml. Twenty-four hours later a blood sugar level of 43 mg. per 100 ml. was obtained, and within an hour the animal died in hypoglycemic convulsions. Thus, at these later injection dates all four animals demonstrated the usual reaction to alloxan.

HISTOLOGY

In this study Hematoxylin-eosin stains were employed routinely. On all pertinent material the *alpha* and *beta* cells of the islands were differentiated by Gomori's modification of his Chromium-hematoxylin-phloxin stain (12).

In order to evaluate the changes found in the pancreas of the young rabbit following the injection of alloxan, alloxan diabetes was produced in an adult animal, and the pancreas from this animal was studied, the animal having succumbed twenty-four hours following injection. Microscopically, aberrations from the normal similar to those described by other investigators (2, 7, 11) were found in the pancreas of this animal (see Fig. 1). The islands showed by both techniques, in varying degrees of severity, the following changes: loss of cording of the cells, loss of cellular outline, coalescence of the cytoplasm into an almost homogeneous debris, pyknosis, and a fading of the nuclei associated with a diminution in the affinity for the basic stain. In the majority of the affected islands were found several cells, apparently undisturbed, which the Gomori stain showed to be *alpha* cells; *i.e.*, the cytoplasm of the *alpha* cells stained a cherry red in contrast to the pale pink cytoplasm of the affected *beta* cells.

Changes in the pancreas of the young rabbits which developed diabetes were comparable to those in the adult rabbit described above (see Figs. 6, 7). In those injected animals in which a diabetic state was not demonstrated there were observed none of the characteristic changes in the pancreas (see Figs. 3, 4, 5). It is known that the *alpha* cells in general are not susceptible to the effects of alloxan (2, 5, 13), which led to the consideration that possibly the islands in these young unsusceptible animals consisted primarily of *alpha* cells. However, when differential stains had been carried out it was found that the majority of the cells in the pancreas of the neonatal rabbit are *beta* cells, and that *alpha* cells are rarely seen.

TABLE IA
Blood Sugar Levels And Weights Of Young Rabbits Injected With Alloxan

TIME AFTER INJECTION

DAY AFTER BIRTH ANIMAL WAS INJECTED		ANIMAL NUMBER	Days														Weeks				Months					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	3	4	5	6	3	5	6	7	8	
2	74-1	B.S. Wt.	165 80					183 147						147 230	147 310	147 310	139 475							185 3790	119 4015	
	74-2	B.S. Wt.	121 62					196 90						141 149	151 220	148 315							112 2560	121 2705		
	74-3	B.S. Wt.	124 60					178 95						154 156	143 225	141 360							135 3280			
3	74-5	B.S. Wt.	137 58	127 64			172 121							166 189		155 270	158 410									
	104-2	B.S. Wt.		122 93	98 123			159 143				175 179	164 207	260		185 311	185 490	158 670								
	105-A1	B.S. Wt.	106 54	117 62	115 59	167 67			123 93				148 128			189 208							175 3540	127 3510		
4	86-2	B.S. Wt.					180 181						174 265				152 430									
	104-4	B.S. Wt.	114 104	104 104	115 120		136 130		173 143	136 156		180		157 228	173 412	165 635										
	105-A4	B.S. Wt.	90 51	125 58	109 76				148 89			168 104					174 216									
	105-A5	B.S. Wt.	106 67	133 71	152 82				154 112			178 118					157 233									

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TABLE IA—Continued
Blood Sugar Levels And Weights Of Young Rabbits Injected With Alloxan

Continued

Blood Sugar Levels And Weights Of Young Rabbits Injected With Alloxan

DAY AFTER BIRTH ANIMAL WAS INJECTED	ANIMAL NUMBER	TIME AFTER INJECTION																												
		Days														Weeks							Months							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	3	4	5	6	3	5	6	7	8	3	5	6	7	8	
9	101-3 B.S. Wt.						198								195	159														
	105-2 B.S. Wt.					308	199			280		315		360	430															
	76-A1 B.S. Wt.					194	205	350*		136				160	167	200														
10	76-A1 B.S. Wt.			150		150	184			148				137																
	76-A2 B.S. Wt.			162		185	192	220		225				275																
	86-5 B.S. Wt.			152		172	184	215		135				145																
11	86-5 B.S. Wt.			157		178	184	215		250				285																
	86-6 B.S. Wt.		155																											
	57-1 B.S. Wt.		156																											
58-3	57-1 B.S. Wt.		180																											
	58-3 B.S. Wt.		220																											
	58-4 B.S. Wt.																													
76-A3	58-3 B.S. Wt.																													
	58-4 B.S. Wt.																													
	76-A3 B.S. Wt.																													
76-A4	58-4 B.S. Wt.																													
	76-A3 B.S. Wt.																													
	76-A4 B.S. Wt.																													
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	76-A4 B.S. Wt.																													

[illegible]

It was noted that within the first two to three weeks after birth the islands appear to be fewer in number than in older animals, and that for a month after birth the islands were of a smaller size in general than in older animals (see Figs. 2, 8). It is interesting to observe that Hughes and Hughes (16) found that in the rat the smaller the islet

TABLE IB
Blood Sugar Levels And Weights Of Control Non-Injected Young Rabbits

ANIMAL NUMBER		TIME AFTER BIRTH																	
		Days														Weeks			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	3	4	5	6
58-5	B.S.			116		143				145						129	155		137
	Wt.			67		88				134						310	445		980
60-4	B.S.						138		144			111				169	154		174
	Wt.						92		117			168				265	405		870
71-A1	B.S.	85		66					126				190			190	184	125	145
	Wt.	38		40					180				320			400	510	710	1020
74-7	B.S.		117	129						166		225			191		129	151	
	Wt.		36	45						129		154			173		225	320	
74-8	B.S.		116	134						186		193			154		164	126	
	Wt.		34	44						109		141			162		225	380	
75-3	B.S.		106					153			95			158		175	142	141	123
	Wt.		45				108	131			210			310		340	470	660	840
76-A10	B.S.									166			131	162	125	158	138		
	Wt.									172			225	260	275	355	465		
98-3	B.S.		94					146				105			200	198	189	179	141
	Wt.		40					155				310			380	460	530	765	950
101-4	B.S.	103	142						110						100	185	207		181
	Wt.	46	54						109						185	315	490		960
105-A3	B.S.		108	116			120		164							126	177		
	Wt.		42	50			115		170							360	505		

B.S. = Blood Sugar; Wt. = Weight.

the larger the mean diameter of the *beta* cells, and that when alloxan was administered in repeated small doses it was the larger *beta* cells that were spared.

DISCUSSION

The results of this experiment indicate that with a dosage of alloxan even larger than that which regularly produced diabetes in the adult

rabbit a demonstrable diabetic state cannot be produced in the young rabbit prior to nine days after birth. Friedgood and Miller, who studied the response of the fetal rat to alloxan, drew the following conclusions: "1) In pregnant rats intravenously injected alloxan passes through the placenta into the fetal circulation within one or two minutes. 2) It does not, however, produce a permanent diabetes in the offspring. 3) The blood sugar values of the fetus parallel at a lower level that of the diabetic mother until parturition" (10). Our results indicate that a refractory state to alloxan exists in the rabbit following birth, and continues thereafter until at least nine days after parturition.

Before discussing possible reasons for this refractory state in the young rabbit, it is interesting to note that several factors have been reported in the literature which influence the production of diabetes by alloxan in the adult animal. Kass and Waisbren (18) have reported that fed rats are less susceptible to the diabetogenic action of alloxan than are those animals which have been starved for forty-eight hours prior to injection, the incidence of hyperglycemia being but 25% in animals whose food had not been withheld in contrast to an incidence of 92% in fasting animals. The same observers also noted, with no explanation, that epinephrine administered immediately before alloxan protected the fasting animals against the diabetogenic action of the drug. Attention has been centered on the rapid destruction of alloxan in the adult animal (14, 21). The present supposition is that the disappearance of alloxan from the blood stream is due to its combination with sulfhydryl compounds. Some evidence for this belief is contained in Leech and Bailey's observation that in rabbits the reduced glutathione of the blood disappeared coincidentally and parallel with the disappearance of injected alloxan (21). Lazarow (20) reported that the intravenous injection of large doses of glutathione or cysteine one to two minutes prior to the injection of a diabetogenic dose of alloxan completely protected rats from diabetes; and that when the sulfhydryl compounds were given one minute after the alloxan injection partial protection occurred, whereas when three or more minutes had elapsed there was no protection.

The explanation of the refractory state of the young rabbit is not evident at present. Two possibilities come to mind. First, the islet

cells of the pancreas at this period of life may be refractory to the drug. Second, alloxan may be destroyed or inactivated in the body immediately after the injection during this period. The evidence mentioned above leads one to consider the possibility that a greater concentration of sulfhydryl compounds may be present in the blood stream or pancreatic islet cells of the young animal than in the adult. In regard to this Needham states that the younger the animal the more glutathione it contains relatively (22). Thompson and Voegtlin found that the concentration of glutathione in the entire rat declines with age both during the embryonic and post-embryonic periods, but that the relationship was not necessarily true for each individual organ (24). In the rabbit this finding was confirmed by Santavy (23). However, the pancreas was not included in these reports.

TABLE II

Susceptibility To Alloxan Of Various Mammalian Species Compared With The Blood Cell Concentration Of Glutathione

SPECIES	MGS. GLUTATHIONE PER 100 GRAMS BLOOD CELLS	MGS. OF ALLOXAN PER KG. OF BODY WEIGHT
Rat.....	53	40 I.V.
Dog.....	79	50-100 I.V.
Rabbit.....	114	100-200 I.V.
Guinea pig.....	151	?*

I.V. = Intravenous.

* See text for explanation.

Goss and Gregory (15) demonstrated in newborn rabbits that sulfhydryl concentration of the entire body is related to nursing. Young which had nursed for forty-eight hours had a mean glutathione concentration of 63 mg. per 100 grams of body weight, while litter mates of the same age which had not nursed had a mean concentration of only 33.94 mg. of glutathione per 100 grams of body weight. Too, these authors found that the glutathione concentration in the newborn varied directly with the adult size of his race; and that the larger embryos within a race have at birth more glutathione than the smaller ones, and this is more the case in races of larger adult size than in those of smaller adult size.

We have collected data relative to the difference in the susceptibility to alloxan of various mammalian species in comparison with the blood

cell concentration of glutathione (Table II). It is interesting to note that the higher the average species blood cell concentration of glutathione, the greater the species resistance to the diabetogenic action of alloxan. The glutathione determinations are from the work of Thompson and Voegtlin (24). The usually efficacious dosages of alloxan for the dog and rabbit are from Goldner (11). The dosage for the rat is from Lazarow (20). Goldner (11) states that the exact dosage for the guinea pig has not been determined, since only a dosage which results in the death of the animal within twenty-four hours produces pancreatic lesions.

That resistance or susceptibility to the diabetogenic action of alloxan does not bear a simple relation to the amount of glutathione in the blood is indicated by the fact that Santavy (23) found that the glutathione content of the blood of the newborn rabbit is approximately one-half that of the adult, and yet our studies show a greater resistance in the newborn rabbit.

Our observations demonstrate that in each case a second injection of alloxan at a later date produced the characteristic response to the drug in animals refractory in the neonatal period. Other observers (5, 16, 19) have reported that in adult animals an animal that has proved refractory to a first dose of alloxan seldom responds to subsequent doses.

CONCLUSIONS

1) A dosage of 300 mg. per kg. of alloxan, which produces diabetes in the adult rabbit, failed to cause diabetes in rabbits when injected before the ninth day of life.

2) In four animals, which did not develop diabetes when injected in the neonatal period with 300 mg. per kg. of alloxan, a later injection of 200 mg. per kg. of alloxan, when the animals were one to six and one-half months old, did produce diabetes in each case.

Acknowledgements: We are deeply indebted to Dr. Peggy Ann Hanson, whose initial studies of the effects of alloxan in the pregnant animal (unpublished) stimulated our interest in this problem.

We wish to express our sincere appreciation to Dr. Arnold R. Rich for his encouragement and invaluable criticism throughout the course of this work.

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DESCRIPTION OF FIGURES

(Photomicrographs by Miss Marjorie Jackson.)

All photomicrographs were taken with blue and yellow filters at the same magnification. The islands illustrated are in each case characteristic of those seen in the respective sections.

FIG. 1. Necrotic island of Langerhans of adult rabbit 69 twenty-four hours after injection with 200 mg. per kg. of alloxan. Hematoxylin-eosin stain.

FIG. 2. Island of Langerhans of normal rabbit 72-4, killed five days after birth. Chromium-hematoxylin-phloxin stain.

FIG. 3. Island of Langerhans of rabbit 105-A3, injected with 300 mg. per kg. of alloxan on the fourth day after birth and killed on the twelfth day after birth. No hyperglycemia; no histological changes typical of alloxan damage. Hematoxylin-eosin stain.

FIG. 4. Island of Langerhans of rabbit 105-A5, injected with 300 mg. per kg. of alloxan on the fourth day after birth and killed on the thirty-second day after birth. No hyperglycemia; no histological changes typical of alloxan damage. Chromium-hematoxylin-phloxin stain.

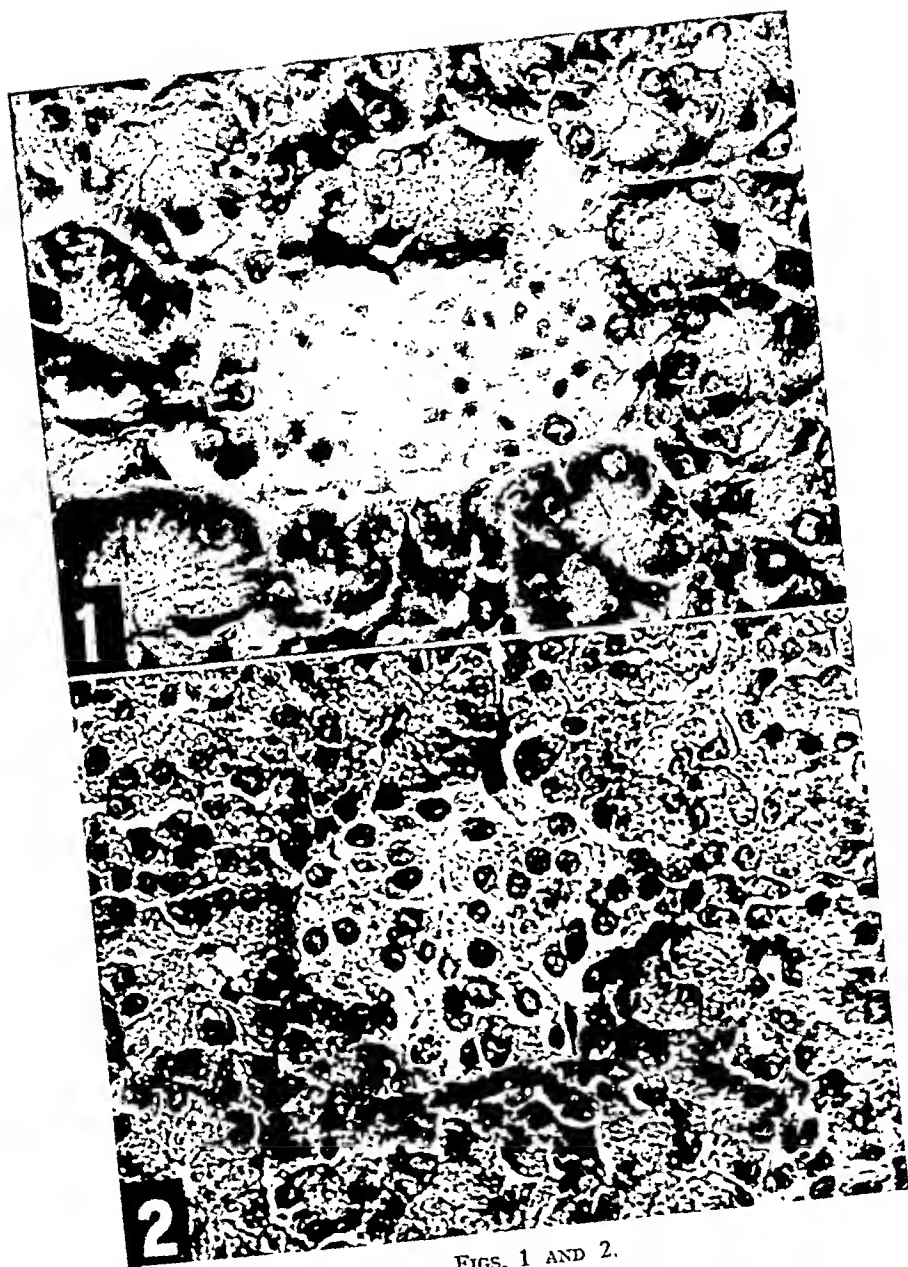
FIG. 5. Island of Langerhans of rabbit 101-B2, injected with 300 mg. per kg. of alloxan on the seventh day after birth and killed on the thirtieth day after birth. No hyperglycemia; no histological changes typical of alloxan damage. Hematoxylin-eosin stain.

FIG. 6. Necrotic island of Langerhans of rabbit 76-A6, injected with 300 mg. per kg. of alloxan on the eleventh day after birth and dead on the following day. No blood sugar determination was made. Hematoxylin-eosin stain.

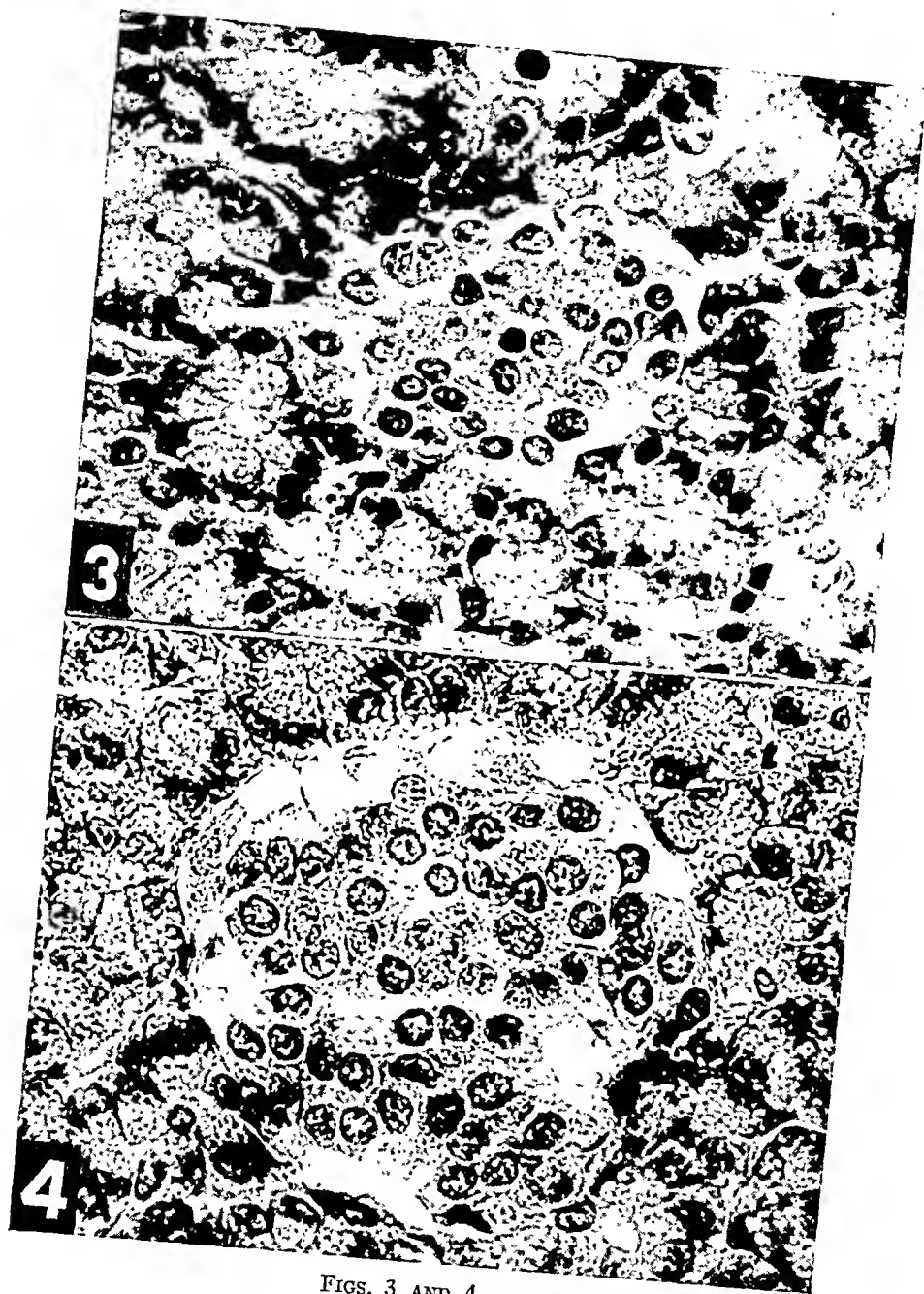
FIG. 7. Necrotic island of Langerhans of rabbit 105-A9, injected with 300 mg. per kg. of alloxan on the seventeenth day after birth and dead on the twentieth day after birth. Hyperglycemia. Hematoxylin-eosin stain.

FIG. 8. Island of Langerhans of normal rabbit 79-4, killed nineteen days after birth. Hematoxylin-eosin stain.

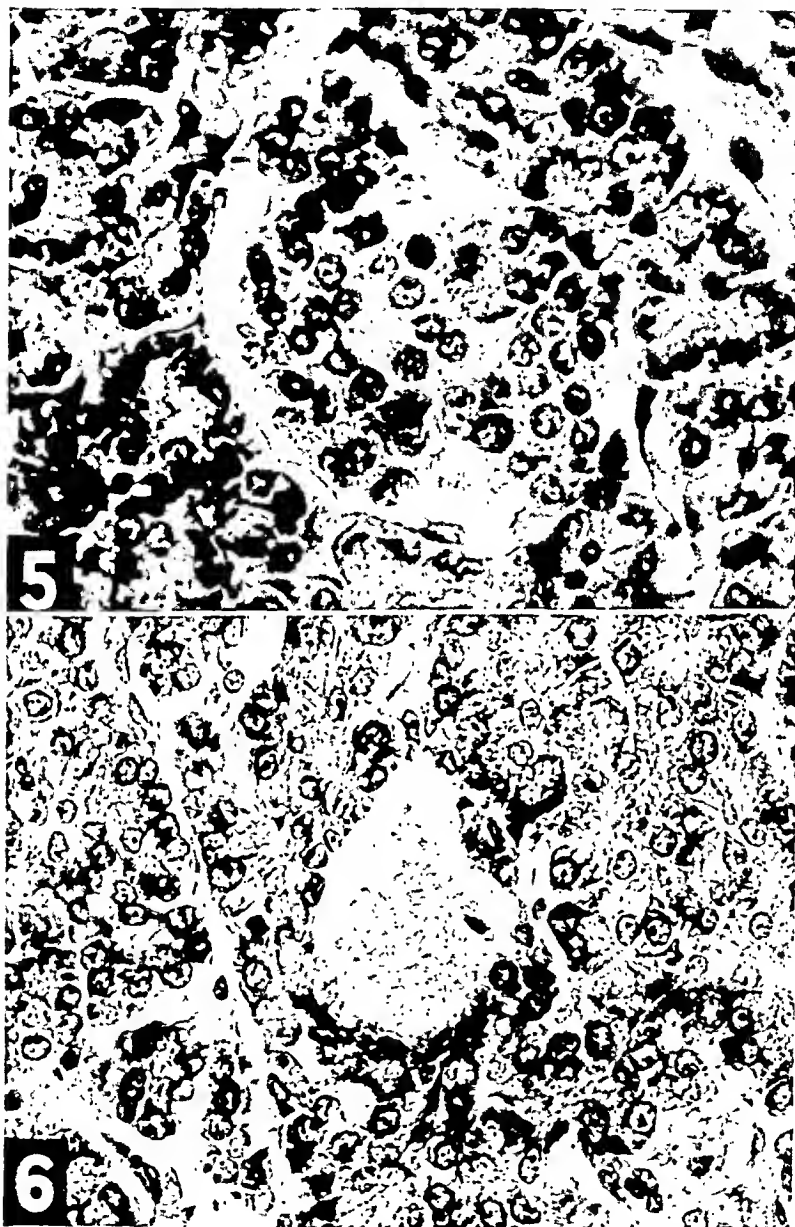
FIG. 9. Necrotic island of Langerhans of rabbit 60-1, first injected with 300 mg. per kg. of alloxan on the sixth day after birth. No hyperglycemia. Reinjecting with 200 mg. per kg. of alloxan on the two hundredth day after birth. The animal died in hypoglycemic convulsions the following day. Hematoxylin-eosin stain.



FIGS. 1 AND 2.



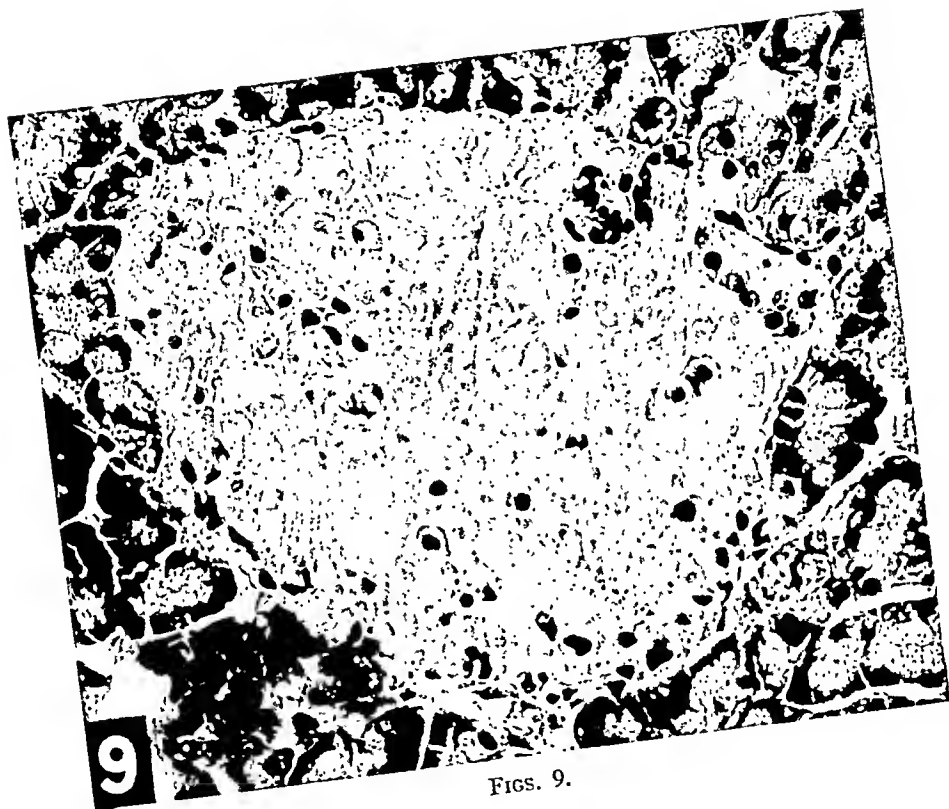
FIGS. 3 AND 4.



FIGS. 5 AND 6.



FIGS. 7 AND 8.



FIGS. 9.

MALIGNANT TUMORS OF THE NASOPHARYNX

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MALIGNANT TUMORS OF THE NASOPHARYNX

The purpose of this paper is to review the clinical characteristics of malignant tumors of the nasopharynx, as they were demonstrated in forty-six cases studied at the Johns Hopkins Hospital between 1925 and 1946.

Nasopharyngeal malignancies have attracted considerable interest because they produce such a perplexing array of local, neurological, otological, and cervical glandular manifestations. They have been studied by many workers, and there are many excellent articles on the subject. The literature has been adequately reviewed by Godtfredson (1) in his comprehensive monograph reporting 453 new cases. The cases to be reported in this paper add nothing new to the wealth of material already published. But repetition is indicated for two reasons. First, these tumors still are misdiagnosed frequently. Second, they demonstrate points of fundamental value, inasmuch as the varied lesions produced can be explained by consideration of the properties of neoplastic growth, the anatomy of the nasopharynx, and the pathological physiological disturbances created by pressure, obstruction, and destruction.

Materials: Forty-six cases of malignant tumor of the nasopharynx studied at the Johns Hopkins Hospital between 1925 and 1946 have been analyzed. In all of the cases considered, a growth could be seen in the nasopharynx. In thirty-three cases, the clinical picture seemed sufficient to establish the diagnosis, even though microscopic evidence was lacking. All of these patients have been examined completely by many observers, all of them have had examination by ear, nose and throat consultants, many have had examination by neurological consultants. Only the data agreed on by all observers have been included. The material has been assembled in the tables that follow. Treat-

ment and mortality are not analyzed because of insufficient data. Godtfriedson covers these subjects adequately.

Results: In Table 1, I have gathered under pertinent headings the salient facts known about each case. Only such detailed analysis can bring out the many different combinations of signs and symptoms presented by the individual case. This compilation of data can be further analyzed as follows.

Types of Tumors: I have not attempted to differentiate minutely the types of tumors involved because the material at hand is inadequate for such study. Such precise work requires serially sectioned autopsy material rather than the small, frequently distorted biopsy material usually obtained. With the aid of the pathology department, the tumors have been divided into the types shown in Table 2. No attempt will be made to consider the relationship between clinical picture and tumor type because of inadequate number of cases for significant statistical study. Godtfriedson brings forth many interesting correlations between tumor type and frequency of various symptoms and signs, but admits that epithelial and mesothelial tumors are both capable of producing similar results.

Incidence: Malignant tumors of the nasopharynx occur at all ages, in negroes and white people, in males and females. Like most malignancies, these occur in highest incidence in the fifth to sixth decade (Table 3). Of these cases 63% occurred in males. For unexplained reasons, males have the disease more commonly than females.

Symptomatology and Signs: These fall into four main groups which may be classified as follows:

1. Nasopharynx: In all cases a visible growth was present. This growth made itself known in many cases by producing obstruction to breathing, discharge (frequently bloody), and pain.
2. Ear: Many cases had some or all of the symptoms and signs of tubal occlusion, including tinnitus, air conduction deafness, feeling of fullness in the ear, and retraction of the ear drums. Acute otitis media was present in one case.
3. Cervical glands: Enlarged cervical glands with signs of metastatic involvement were common. Sometimes these were present on one side, more often they were bilateral. The glands between the tip of the mastoid and the sternocleidomastoid muscle were most frequently involved.

TABLE 1-Continued

CASE AND SERIAL NUMBER	COLOR	SEX	AGE	1ST SYMPTOM	INTERVAL FROM TOX TO ADMISSION	CERVICAL GLANDS	NASO-PHARYNX*	EAR	CRANIAL NERVES INVOLVED	X-RAY SKULL	REMOTE METASTASES	OTHER	TYPE TUMOR
23. 391489	W	F	45	Postnasal drip	1 1/2 years	R and L	Obstruction. Bloody discharge	Both ears tinnitus AC deafness and drum retraction	R and L 6, 10, 12,	Displacement of air column	None demonstrated	General headache, fits of weakness, feeling hot, defecating and urinating	Squamous cell, carcinoma
24. 331009	C	M	44	Pain left temple, ear and nose	1 year	0	0	Left ear AC deafness	0	Displacement of air column. Clouding of left antrum and sphenoid	None demonstrated	0	Squamous cell, carcinoma
25. 168735	C	M	57	Nasal obstruction	1 year	R and L	Obstruction. Bloody discharge	Left ear AC deafness and drum retraction	L 5, 7, 11	Displacement of air column. Destruction of base of skull. None obtained	None demonstrated	0	Squamous cell, carcinoma
26. U46131	C	M	52	Sore throat and swollen neck glands	4 mos.	R and L	Bloody discharge	Left ear AC deafness	0	Displacement of air column	None demonstrated	0	Squamous cell, carcinoma
27. 324885	W	M	54	Obstruction left side of nose	8 mos.	R and L	Obstruction. Bloody discharge	0	0	Displacement of air column	None demonstrated	0	Carcinoma
28. A16216	W	M	4	Pain right side of face	6 wks.	R and L	Obstruction. Bloody discharge	Right ear AC deafness	R 3, 4, 5, 6	Displacement of air column erosion of nose bones	None demonstrated	0	Lymphoepithelioma
29. 164637	C	F	4	Sore throat	4 days	R and L	0	Left ear otitis media	L 5, 6, 7	Not obtained	Nystagmus. EEG showed irritative focus on right side	Rhabdomyoma	Sarcoma

30. U5314	W	F	69	Pain right side of face	2 years	R and L	0-	Right ear tinnitus AC deafness	R 1, 5, 7, 9	Negative	None demonstrated Enlarged	0	Sarcoma
31. U191363	W	M	53	Tinnitus right ear	1 year	R and L	Obstruction. Bloody discharge	Right ear tinnitus AC deafness and drum retraction left ear drum retraction	0	Not obtained	Enlarged spleen, axillary and inguinal nodes	0	Lymphosarcoma
32. U142011	W	F	25	Low lumbar pain	1 year	R and L	Bloody discharge	Feeling of fullness in both ears	0	Not obtained	Abdominal mass, ascites, and hydrothorax	0	Lymphosarcoma
33. U2111	C	F	50	Painless swelling left cervical glands	4 mos.	R and L	Bloody discharge	Both ears tinnitus AC deafness and drum retraction	0	Not obtained	Retropetitioned axillary and inguinal nodes	0	Round cell sarcoma
34. U73621	W	F	38	Tinnitus right ear	1 year	R and L	Postnasal drip that is often bloody	Right ear tinnitus AC deafness fluid level	R 5, 6, 7	Negative	None demonstrated	Vomiting	No biopsy
35. U72595	C	M	53	Generalized headache	3 years	R and L	Bloody discharge	0	0	Not obtained	None demonstrated	0	No biopsy
36. U61870	W	M	48	Pain left temporal region	9 mos.	L	Bloody discharge	Left ear tinnitus and AC deafness	L 3, 5, 7	Not obtained	None demonstrated	0	No biopsy
37. U58297	C	M	51	Painless swelling of left cervical nodes	2 years	R and L	0	Left ear tinnitus AC deafness and drum retraction	L 5	Not obtained	None demonstrated	0	No biopsy
38. U16350	W	M	25	Pain right side neck, head, and face	14 wks.	R and L	0	Right ear tinnitus AC deafness and drum retraction	R 2, 5, 6, 7, 9, 10, 12	Not obtained	None demonstrated	Projectile vomiting	No biopsy
39. U51250	W	F	45	Painless swelling of right cervical nodes	2 years	R and L	Bloody discharge	Right ear tinnitus AC deafness and drum retraction	R 5, 9, 10	Not obtained	None demonstrated	0	No biopsy
40. U60527	C	F	18	Pain left side neck, head, and face	4 mos.	L	0	Both ears AC deafness	L 5, 6, 9, 10, 12	Not obtained	None demonstrated	Vertigo, staggers to right	No biopsy

TABLE 1-*Concluded*

CASE AND SERIAL NUMBER	COLOR	SEX	AGE	1ST SYMPTOM	INTERVAL FROM 1ST SYMPTOM TO ADMISSION	CERVICAL GLANDS	NASOPHARYNX*	EAR	CRANIAL NERVES INVOLVED	X-RAY SKULL	REMOTE METASTASES	OTHER	TYPE TUMOR
41. U95598	W	F	62	Pain right temporal region	10 mos.	0	Bloody discharge	Right ear AC deafness and tinnitus	R 5, 9, L 8, 10	Destruction of floor of anterior fossa. Involvement of posterior ethmoid and sphenoid sinuses and of petrous bone. Destruction of right sphenoid	None demonstrated	Vomiting	No biopsy
42. 122111	W	F	61	Pain right occipital facial region	14 mos.	R and L	Bloody discharge	Right ear AC deafness and drum retraction	R 5				
43. U24565	W	F	16	Nasal obstruction	1½ years	R and L	Non-bloody discharge	Both ears AC deafness and drum retraction. Left ear tinnitus	L 3, 4, 9 10	Not obtained	None demonstrated	0	No biopsy
44. 193073	W	F	38	Decreased vision right eye	1 year	0	Bloody discharge	Right ear tinnitus and AC deafness	R 2, 5, 6, 9, 10, 12	Destruction of right middle fossa	None demonstrated	Left suboccipital headache	No biopsy
45. U72431	W	M	53	Right ear AC deafness and feeling of fullness	3 mos.	0	0	Right ear AC deafness and drum retraction	0	Bones of thorax and extremities. Lungs	Generalized headache worse in occipital region	No biopsy	No biopsy
46. 295231	W	F	54	Right ear AC deafness	4 years	0	Bloody discharge	Right ear AC deafness	R 5, 6, 7, L 2	Destruction of middle fossa, right sphenoid sinus and right orbit	None demonstrated	Vomiting Proptosis right eye	No biopsy

* In every one of these forty-six cases a visible growth was present in the nasopharynx. Only findings other than visible growth are mentioned therefore in this column.

4. Neurological: Symptoms and signs of involvement of cranial nerves were frequently present. Pain and anaesthesia in appropriate

TABLE 2

The types of tumors found in the 46 cases of malignant tumor of the nasopharynx

<i>Epithelial Tumors</i>	
Squamous cell carcinoma.....	14
Carcinoma.....	11
Adenocarcinoma.....	1
<i>Mesothelial Tumors</i>	
Lymphosarcoma.....	3
Sarcoma.....	2
Rhabdomyoma.....	1
Lymphoepithelioma.....	1
<i>Non Biopsied Tumors</i>	13

TABLE 3

The age of the patients in 46 cases of nasopharyngeal malignancy

AGE	NUMBER OF CASES
1-10	2
10-20	3
20-30	5
30-40	5
40-50	12
50-60	11
60-70	7
70-80	1

TABLE 4

Classification of the type of first symptom in forty-six cases of malignancy of the nasopharynx

Nasopharynx	10 cases
Ear.....	10 cases
Cervical Gland.....	9 cases
Neurological.....	14 cases
Pain Lumbosacral Region.....	2 cases
Generalized Headache.....	1 case

distribution marked involvement of sensory nerves. Weakness, paralysis, and atrophy of appropriate muscles marked involvement of motor nerves.

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The first symptom was in almost equal proportion referred to one of the four main groups (Table 4). Where the first symptom was neurological, in 85% of the cases it was fifth nerve pain. In two cases, pain from metastases in the lumbosacral region produced the first symptom. In one case generalized headache, probably on the basis of invasion of the base of the skull, was the first symptom.

TABLE 5
The interval between the first symptom and admission in 46 cases of nasopharyngeal malignancy

INTERVAL BETWEEN FIRST SYMPTOM AND ADMISSION	NUMBER OF CASES
0- 4 months	12
4- 8 months	3
8-12 months	17
1- 2 years	10
2- 3 years	2
3- 4 years	2

TABLE 6
The incidence of various types of symptoms and signs on admission in forty-six cases of malignancy of the nasopharynx

Nasopharynx (exclusive of visible growth which was present in all cases)	25 cases = 52%
Ear	43 cases = 93%
Cervical Gland	36 cases = 78%
Neurological	31 cases = 67%
Headache	5 cases = 11%
Vomiting	5 cases = 11%

The interval between the first symptom and admission to the hospital varied considerably, as may be seen in Table 5. In many of these cases, the delay in great part was due to misdiagnosis.

On admission to the hospital, the patients almost always had overt manifestations of their disease, falling into several of the main groups already discussed (Table 6). The neurological lesions were very varied (Table 7). Frequently, there were multiple lesions. The fifth nerve was the one most frequently involved. In one instance (case 20) a true Horner's syndrome was present. Headache, usually occipital, which did not seem to be related to cranial nerve involvement, was observed in five instances. Projectile vomiting was seen in five instances.

Special studies: Either the throat mirror or the nasopharyngoscope

was necessary for adequate visualization of the tumor. Roentgenograms of the skull were obtained in only twenty-one of the forty-six

TABLE 7

*The distribution of neurological involvement in the cases after full development.
31 of the 46 cases had some manifestations of neurological involvement.
Frequently, more than one nerve was affected in a single case.*

CRANIAL NERVE INVOLVED	NUMBER OF CASES
I	1
II	6
III	6
IV	3
V	27
VI	13
VII	10
VIII	1
IX	13
X	11
XII	8
Cervical Sympathetic	1

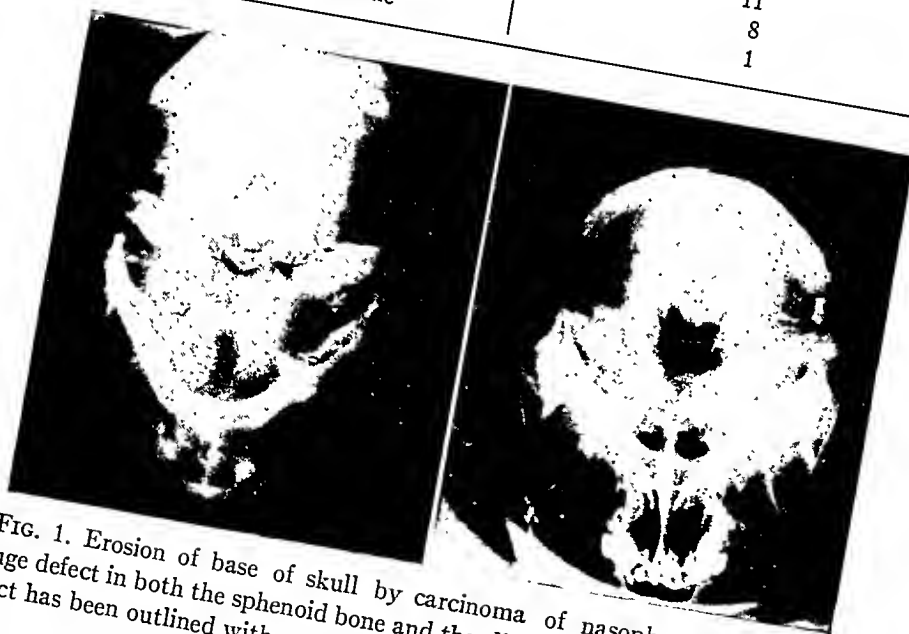


FIG. 1. Erosion of base of skull by carcinoma of nasopharynx. There is a huge defect in both the sphenoid bone and the clivus of the occipital bone. This defect has been outlined with arrows.

cases. They proved to be of considerable help diagnostically. Definite erosion of the base of the skull, of the type shown in Figure 1,

was demonstrated in twelve instances. The soft tissue shadow of the tumor displacing the nasopharyngeal air column, as in Figure 2, was demonstrated in seven instances. The roentgenograms showed no evidence of tumor in seven instances. Had anteroposterior and lateral films of the skull and stereoscopic films of the base of the skull been taken routinely in all cases, they probably would have revealed evidence of tumor in a large percentage of the cases.



FIG. 2. Displacement of air column by carcinoma of nasopharynx. The wide air column which normally traverses the nasopharynx and can be seen overlying the superior rami of the mandible has been obliterated by a soft tissue mass which has been outlined by arrows.

Autopsy Examination: Seven of the forty-six cases were autopsied. In two autopsied cases, 12 and 33, the findings were an ulcerated tumor in the nasopharynx with metastases in cervical lymph nodes. In the first case, the tumor was a squamous cell carcinoma; in the second, a round cell sarcoma. The round cell sarcoma had also extended to retroperitoneal, axillary, and inguinal lymph nodes. In both of these cases, the autopsy findings confirmed the clinical impression. There were no neurological manifestations. There was no demonstrated invasion of the base of the skull.

Five autopsies were performed on cases with neurological manifestations. Unfortunately, in no case were the cranial nerves dissected out and special stains applied in order to determine the point of involve-

ment. However, in all cases, tumor infiltrated the bones at the base of the skull in areas where the nerves clinically involved might well have been affected. These data are summarized in Table 8.

Comment: The clinical picture presented by these tumors is explained by the following considerations.

The tumors are surface growing neoplasms, which tend to overgrow their blood supply and become necrotic. Secondary ulceration and infection frequently develop. A certain amount of hemorrhage, discharge, and local pain is therefore to be expected. Moreover, such a growth is a space occupying lesion capable of producing nasal obstruction when it becomes large enough.

These growths start in the neighborhood of the Eustachian tube orifices, and, by direct extension and the production of secondary edema, they readily occlude these orifices. Inadequate drainage of the middle ear results, and there follows the usual sequence to the blockade of any air filled space in the body—resorption of air, accumulation of secretions, and susceptibility to infection. Resorption of air from the middle ear produces tinnitus, causes a feeling of fullness in the ear, and leads to deafness. Accumulation of secretion causes the fluid level and bulging drum observed in case 34. Infection produces otitis media as in case 16.

The lymphatic drainage of the nasopharynx is rich and these tumors are very malignant. Early extension via lymphatics would be expected. The main drainage is to cervical nodes, those between the tip of the mastoid and the sternocleidomastoid muscle being the first major way stations. These nodes are usually the first to be involved.

The nasopharynx lies in close proximity to many cranial nerves. The cranial nerves emerge from the brain stem and penetrate the base of the skull which forms the roof of the nasopharynx. The roentgenogram and autopsy evidence indicate that the base of the skull is frequently invaded by the tumor. Such invasion is usually most extensive in the areas where the clinically involved nerves might be affected. The fact that the foramen lacerum and the Eustachian tube allow an easy gateway to the region of the petrous tip probably accounts for the high incidence of involvement of the fifth nerve and the nerves to the extra-ocular muscles. The cervical sympathetic supply to the eye may be interrupted either here or in the neck (by cervical metastasis).

TABLE 8

Significant findings at autopsy in cases with neurological involvement

CASE	CLINICAL NEUROLOGICAL INVOLVEMENT	ROENTGENOGRAM OF SKULL	SIGNIFICANT AUTOPSY FINDINGS
8	L. 5,6,7,9,10	None taken	Carcinoma of the nasopharynx. The growth had invaded the petrous portion of the temporal bone, involving it completely. It had spread beneath the dura where involvement of the cranial nerves occurred. The left temporal lobe was penetrated along the VIII nerve.*
20	L. 2,5,6	Destruction of base of skull	Squamous cell carcinoma of the nasopharynx. Tumor was found invading the base of the skull. On the left lateral aspect of the sphenoid bone, the tumor had apparently completely invaded the dura and was growing along the inner surface about the cranial nerves, especially the fifth which was surrounded by tumor. Tumor had destroyed the bone beneath the posterior portion of the sella and compressed the hypophysis. The pons was invaded.
28	R. 3,4,5,6	Almost complete destruction of the sphenoid bone.	Rhabdomyoma of nasopharynx. Erosion of bones at base of skull. Extension to right middle cranial fossa and to right cerebello-pontine region. Compression of aqueduct and fourth ventricle with enlargement of third and lateral ventricles. Invasion of sphenoidal sinus, floor of the sella, and hypophysis. Extension to right orbit and right submaxillary region.
29	R. 5,6,7	None taken	Sarcoma of the nasopharynx. Erosion into the base of the skull. Tumor was found all through the petrous portion of the left temporal bone and in the surrounding soft tissues. It did not break into the cranial cavity. Cervical metastases.
30	R. 1,5,7,9	Negative	Sarcoma of the nasopharynx. Tumor pushed into the right antrum and into the sphenoidal sinus, appearing through the right part of the sella turcica. It extended up along the right carotid artery. It invaded surrounding tissues vigorously.

* Autopsy performed at Hartford Hospital, Hartford, Connecticut by Dr. Kendell.

ses). Pressure on nerves with resulting irritation and ablation phenomena easily explains all of the symptoms noted in the appropriate distribution of the involved cranial nerves. In one instance, case 8, actual invasion of the temporal lobe took place with a resulting agraphia.

SUMMARY

The clinical picture presented in forty-six cases of malignancy of the nasopharynx has been analyzed. An attempt to explain this picture on the basis of the anatomy of the nasopharynx, of the properties of neoplastic growth, and the disturbances created by pressure, obstruction, and destruction, has been made.

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THE TREATMENT OF MILIARY TUBERCULOSIS WITH PROMIZOLE*

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Within recent years a number of chemical agents have been used in the treatment of tuberculosis. Encouraging results have been reported with the use of promine and diasone (1, 2), but treatment of tuberculosis in adults with promizole has not been reported favorably (3). However, promizole produced very encouraging results in experimental tuberculosis of small animals (4). We were given the opportunity on the Children's Medical Service at Bellevue Hospital to attempt the treatment of tuberculous children with this drug. A preliminary report of this study has already been published (5).

We decided to try promizole first in that form of tuberculosis which has the worst prognosis and treated five successive cases of meningitis with no beneficial effects that could be determined. The children died six to twenty-five days after treatment was initiated and postmortem examination of the brains in two cases showed no demonstrable retardation of the disease.

Acute generalized hematogenous tuberculosis was then selected for treatment with promizole because the course of this disease is more prolonged than that of meningitis thus allowing more time for action of a drug. The prognosis of this form of tuberculosis is almost as serious as that of tuberculous meningitis. Engel (6) saw only one recovery in fifteen years in a large pediatric service. Wallgren (7) reported 84 cases of miliary tuberculosis seen over a period of fifteen years with recovery in five. We have not seen a permanent recovery from acute miliary tuberculosis on the Children's Medical Service of Bellevue Hospital during an observation period of 25 years.

During a period of over 17 years prior to the beginning of this study in June 1944, 102 cases of generalized tuberculosis of miliary or slightly

* Aided by grants from the National Tuberculosis Association and from Parke Davis & Company.

larger size were seen. Death occurred in 88 of these cases within three months after the diagnosis of miliary tuberculosis was established and only three children survived for more than a year. In two of these latter cases the disease progressed slowly until death occurred from meningitis in one case, and from chronic pulmonary tuberculosis in the other. The third case, a negro boy two years old was the only one who showed definite recession of the miliary tubercles on x-ray. He died of acute tuberculous pneumonia and streptococcic pericarditis 23 months after the diagnosis of miliary tuberculosis was first established. On postmortem examination the miliary tubercles in the lungs, liver and spleen were in varying stages of fibrosis, hyalinization and calcification, although some caseous tubercles were present (8).

Every case in this series showed roentgen evidence of mottling throughout both lung fields, and manifest primary tuberculosis was present on x-ray in 91%. In more than 25% of the cases, the development of miliary tuberculosis occurred while the children were under observation for primary tuberculosis. In practically every child fever accompanied, and usually preceded, the appearance of the miliary shadows on x-ray but there was no abrupt change in general condition and nothing but the fever to date the onset of this complication. Moreover in this group, where the time of onset of miliary tuberculosis was known, as well as in our entire group of 102 cases, enlargement of the spleen and of the superficial lymph nodes was not a constant finding occurring in only 60% of the cases later proven by autopsy to have generalized tuberculosis.

Our past experience therefore leads us to believe that miliary pulmonary tuberculosis is a disease of very grave prognosis, that it rarely clears even temporarily and that in children it is almost invariably part of the clinical picture of generalized miliary tuberculosis.

TREATMENT OF ACUTE MILIARY TUBERCULOSIS WITH PROMIZOLE

DOSAGE

In our first cases we gave an initial dose of 1 gm. daily and increased the dosage rapidly to the point of toxicity. In our more recent cases we have begun with 1 gm. daily and increased slowly, checking with the blood levels, until a daily dose up to 5 gms. was reached. We have attempted to achieve a blood level of promizole of 2 to 3 mgms. % since

we secured apparent therapeutic effects at this level. In two of our early patients, acting on the advice of Dr. Medlar, we reduced the dose to 1 gm. daily after treatment for six months with larger doses and in another case the dose was reduced after two months because of leucopenia. It has been possible to maintain adequate blood levels on this low dosage. We plan to diminish the dose in new cases after we have

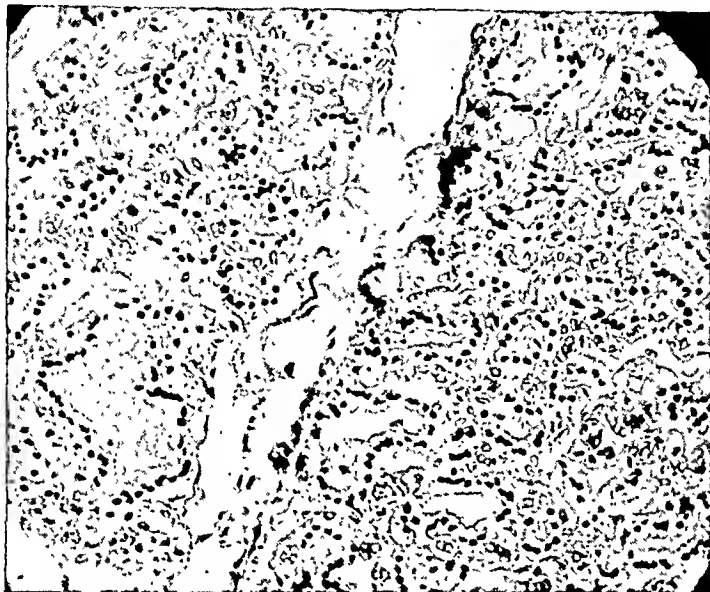


FIG. 1. Thyroid gland of a child who died of miliary tuberculosis after treatment with 43 gms. of promizole during a period of 34 days. Only a rare acinus contains colloid. Tall columnar epithelium lines most of the follicles. Similar changes have been described in guinea pigs treated with promizole.

clinical evidence of the effect of the drug either in diminution of the mottling on x-ray or in toxic effects such as leucopenia.

TOXIC EFFECTS

Vomiting has been severe in only one case and ceased after the drug was temporarily discontinued. The fluid intake of this child had been low and she tolerated the drug well when administration was resumed.

Blood. Leucopenia occurred in two cases after three weeks and after four months of treatment. The lowest white blood cell count was 2,780 with 50% polymorphonuclear cells; after temporary withdrawal of the drug the count rose. One child, after nine days of treat-

ment showed a fall in hemoglobin to 8 gm. per 100 cc. and a red blood cell count of 2,960,000 per cu.mm. Following one transfusion his red



FIG. 2. P. C., Case I, a 2 year old girl, showing enlarged nipples which appeared after 7 months of treatment.

blood cell count and hemoglobin remained within normal limits on continued administration of the drug.

Cyanosis appeared as early as a few hours after the first dose of promizole and was persistent in two children. Methemoglobin was found in the blood by photospectrometry even when cyanosis was not noted clinically.

Skin. Vulval pruritis lasting for a week occurred in one case.

Promizole had been discontinued for one week and the symptoms began several hours after the medication was resumed.

Jaundice. One boy with peritonitis and enlarged liver had icteric sclerae and skin and an icterus index of 20 for a week. We can only speculate whether the icterus was due to liver damage from tuberculosis or to the medication.



FIG. 3. R. C., Case II, a 5 year old girl, showing enlargement of breasts after 20 months treatment.

Thyroid Gland. A definite increase in size appeared in all our cases two to five months after the drug was started. Withdrawal of the drug or the administration of desiccated thyroid gland caused marked decrease in size of the gland. This effect of promizole has been previously described (9). On autopsy in animals hyperplasia of the thyroid gland with markedly diminished colloid secretion was reported. One of our cases who died after 34 days of treatment showed a similar reaction in his thyroid gland (Fig. 1). This action of promizole appar-

ently resembled the effect of sulfonamides and thiourea on the thyroid gland (10). In the three cases treated for more than two years, the rate of development of the carpal centers has been within normal limits.

Sex Maturation. The three cases treated for more than two years all showed secondary sex characteristics. The younger girl, Case I, developed enlarged nipples (Fig. 2) and a few labial hairs after seven months of treatment when she was less than three years old. Urine assay at this time showed a slight increase in the excretion of 17 ketosteroids; 1.9 mgms. daily.¹ Case II showed nipple enlargement after twenty months of treatment and her urine assay showed excretion of 3.1 mgms. daily of 17 ketosteroids. The breast tissue in this child at the age of five years is palpable and visible (Fig. 3). The boy has no visible change in his genitalia but pubic hair is present at the age of eight years.

CASES OF ACUTE GENERALIZED MILIARY TUBERCULOSIS TREATED WITH PROMIZOLE

We have treated eleven children with acute miliary tuberculosis with promizole. All showed evidence of primary pulmonary tuberculosis as well as mottling of miliary or slightly larger size throughout both lung fields.

Three of these cases have been treated for less than six months and while all are doing well it is too early to evaluate results of therapy. Three cases were treated for less than a month. These comprise a four months old white boy (W. L.) who died 16 days after treatment was begun; a 16 months old negro boy (R. G.) who was treated intensively for 12 days and then removed from the hospital against advice. This boy is alive two years later in a hospital for tuberculous children and still shows evidence of miliary tuberculosis on x-ray. It is impossible to place his case in either the treated or untreated group. The third case (V. B.) was a white girl four months old treated for 26 days, whose medication was stopped because of a shortage of the drug. She died of tuberculous meningitis, showing the first symptoms five weeks after medication was discontinued.

Five consecutive cases of acute miliary tuberculosis were treated for more than a month. Two of these cases died, one of meningitis

¹ Urine assays for 17 ketosteroids were done by Dr. Konrad Dobriner.

after $4\frac{1}{2}$ months of treatment and the other after receiving promizole for seven weeks. Three children are alive and apparently free from miliary tuberculosis over two years after treatment was begun. A brief summary of the two cases who died follows:

R. L. Porto Rican male was first seen when six months old with a possible pneumonia following mastoidectomy. X-ray revealed a clouding of the right upper lobe and fine mottling throughout both lungs. Miliary tuberculosis was diagnosed



FIG. 4. (A) P. C., Case I. X-ray taken May 1944 shortly before treatment showing mottling throughout both lung fields.

and promizole begun at the age of seven months in dosage of 0.5 gm. daily. The infant at this time weighed 13 lbs. and was acutely ill with high fever and a constant paroxysmal cough. There was some clinical improvement at first, fever was lower and on some days the temperature was normal; anorexia disappeared and there was a small weight gain. But the primary focus and the disseminated mottling increased steadily in size. Fever recurred and the child died after $4\frac{1}{2}$ months of continuous therapy with promizole.

E. B. negro male two years old was admitted to Bellevue Hospital December 1944 with a history of two months illness and x-ray evidence of miliary tuberculosis and primary pulmonary tuberculosis. On admission his temperature ranged from 102°F. to 104°F. daily, he was acutely ill, and had a paroxysmal cough. His liver was felt almost at the level of the umbilicus and the spleen was palpable. One

gram promizole daily was first given December 19 and increased to two grams daily by January 10, 1945. Medication was suspended January 13, 1945 because of extreme distension but started again on January 24 and continued until death on February 9. Serial x-rays showed progressive increase in size of the hematogenous tubercles and cavitation in the primary focus. Autopsy revealed caseous primary tuberculosis and tuberculous pneumonia and empyema, miliary tuberculosis of lungs, liver, spleen, kidney and pericardium and early tuberculous menin-



FIG. 4. (B) P. C., Case I. X-ray taken March 1946 showing marked clearing of miliary shadows, although the primary focus is still visible in the third and fourth left interspaces. Note the annular shadow in the third interspace interpreted as a cavity.

gitis. The thyroid gland was normal in size but on microscopic examination showed an almost complete absence of colloid and other changes similar to those described by Feldman (4) in guinea pigs treated with promizole (Fig. 1).

The three cases who have survived acute miliary tuberculosis are reported in greater detail:

Case I. P. C. negro girl was admitted February 1944 at the age of two years with an excavating primary tuberculosis of the left lower lobe and miliary seeding throughout both lungs (Fig. 4-A). Irregular fever to 102°F. was present for a

month before admission and for five weeks after entering the hospital. She had lost weight but was in fair condition, weighing 22 lbs. and did not look ill. The liver was felt two finger breadths below the costal margin; the spleen was not palpable. There was a general slight enlargement of the superficial nodes. During the $3\frac{1}{2}$ months following admission her general condition improved and she gained weight. Erythrocyte sedimentation rate fell from 28 mm. in an hour to 14 mm. (Cutler Method) and white blood cell count from 20,000 to 12,000. However, the mottling on x-ray became larger, the primary cavity more definite and

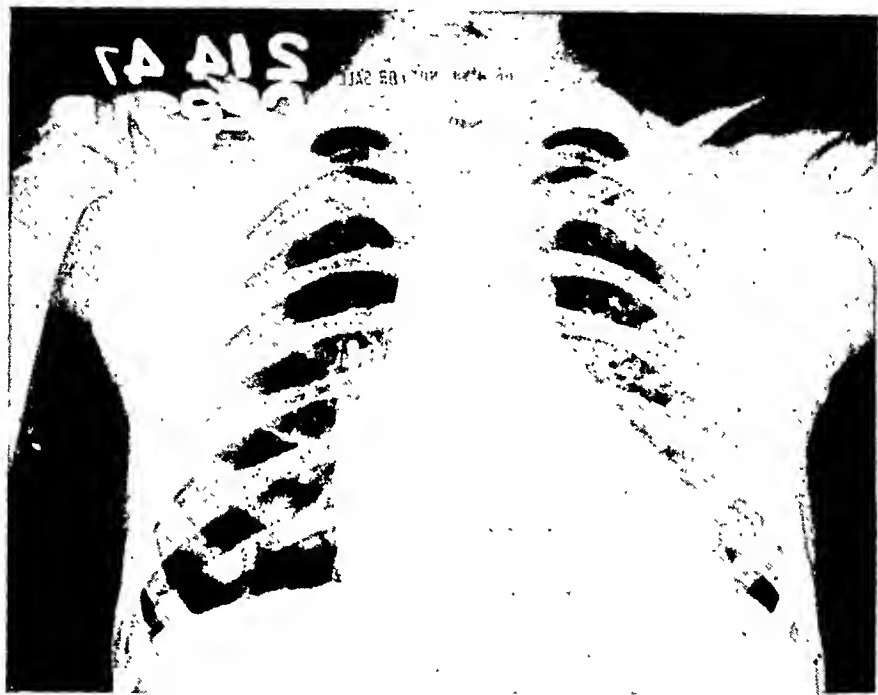


FIG. 4. (C) P. C., Case I. X-ray taken February 1947, showing clear lung fields except for faint clouding in third and fourth left interspaces, remains of primary focus.

her spleen became palpable. Promizole 3 gms. daily was started after $3\frac{1}{2}$ months of observation on June 6, 1944. Ten weeks later in August 1944 the mottlings on x-rays first appeared smaller. In October she had a fever of 102°F . and diminished breath sounds with moist rales were found over the left chest posteriorly. X-ray showed an extension of the primary lesion and shortly after this acid fast bacilli were found in the gastric contents for the first and only time. The dose of promizole was increased to 4 gms. daily. In November the miliary lesions were less evident on x-ray and by January 1945 the mottling was barely visible, the rales had disappeared and the spleen was no longer palpable. The cavity in the primary focus was last seen on August 30, 1946 (Fig. 4-B). At the present time there is still a

faint area of clouding at the site of the primary lesion and small calcifications are visible at the base of the left lung and in the spleen, but no mottling is visible in the lung fields (Fig. 4-C). This child has to remain in the hospital for social reasons but is now apparently well three years after the onset of miliary tuberculosis.

Case II. R. C. negro girl was admitted in May 1944 at the age of four years with an extensive excavating primary tuberculosis of the left upper lobe and with mottling of miliary size throughout both lungs (Fig. 5-A). She had been ill with



FIG. 5. (A) R. C., Case II. X-ray taken June 1944 before treatment, showing clouding in left lung to second rib, with enlargement of right root and mediastinum widened to right. Mottling of miliary size is seen throughout rest of both lung fields.

an irregular temperature to 103°F. for two months before admission but did not look acutely ill on admission and was well nourished, weighing 39 lbs. Dullness and inconstant moist rales were heard over the left upper anterior lung field. The liver edge was one finger breadth below the costal margin, the spleen was easily palpable and there was general enlargement of the superficial lymph nodes. Promizole 3 gms. daily was begun on June 6, 1944. Within 24 hours she began to vomit, cyanosis was noted and on June 12 promizole was stopped because of delirium and choreiform movements of the extremities. After this condition subsided, administration of promizole was resumed with 2 gms. daily and later the dosage was increased to 2½ gms. There was no return of symptoms of toxemia. Six weeks

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after beginning promizole there was roentgen evidence of increase in size of the primary focus with definite cavitation. The mottlings also appeared larger at this time. The temperature now remained below 101 and the child began to gain weight. The mottling on x-ray first appeared smaller in October 1944, 4½ months after treatment was initiated, although the primary focus remained unchanged and acid fast bacilli were recovered at this time from the gastric contents. At this time the red blood cell count was 3,000,000 per cu.mm. and the white blood cell count reached 3,800 per cu.mm. in November. Promizole was discontinued for about a month and then 1 gm. daily was begun in December and this dose has been

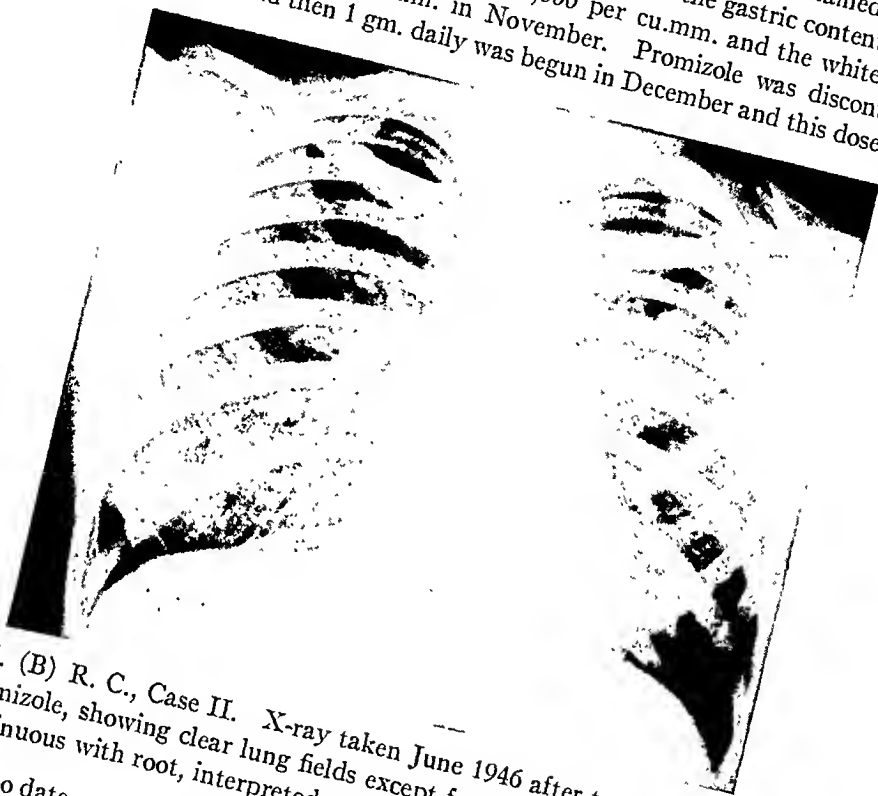


FIG. 5. (B) R. C., Case II. X-ray taken June 1946 after two years treatment with promizole, showing clear lung fields except for clouding in second left interspace continuous with root, interpreted as remains of primary focus.

continued to date and the blood count has remained normal. The area of clouding in the left upper lobe had diminished markedly by April 1945 and the miliary seeding was barely visible, the cavity had disappeared and no rales could be heard. The spleen was just palpable and the temperature was normal except for a very occasional rise to 100.5°F. The child was sent home and has remained well. Since January 1946 no mottling has been visible on x-ray (Fig. 5-B). In January 1947 a faint clouding in the second left interspace and a moderate widening of the mediastinal shadow to the left were the only pathological roentgen findings.

Case III. J. A. a Porto Rican boy six years old was admitted to Bellevue Hospital on August 31, 1944. The first diagnosis of miliary tuberculosis had been made three months previously when he had high fever, enlarged liver and spleen,

a positive tuberculin test and x-ray evidence of mottling throughout both lung fields in addition to enlarged right bronchopulmonary nodes (Fig. 6-A). Two weeks prior to transfer to Bellevue he had developed a pleural effusion on the right side which was subsiding at the time of admission. Within the first month at Bellevue he developed a left sided pleural effusion and following this, abdominal tenderness with marked enlargement of liver and spleen. Promizole was started on October 14, 1944 in dosage of 3 gms. daily. He vomited at intervals, was



FIG. 6. (A) J. A., Case III. X-ray taken October 1944 before treatment showing left pleural effusion, enlarged right root and mottling of miliary size throughout both lungs.

cyanotic and dyspneic, and lay constantly with his knees flexed. Promizole was discontinued after 23 days because the white blood cells were only 3,800 per cu.mm. but was begun again in 1 gm. dosage after an interval of a month and has been continued to date. The abdominal symptoms disappeared after one month but there was at the same time an increase in the mottling on x-ray. In addition, culture of the urine on December 5, 1944 showed tubercle bacilli after microscopic examination of urine constantly showed a few white blood cells. The positive urine cultures continued for ten months although retrograde pyelograms were

normal. Urine cultures have been negative since October 1945 although white blood cells continue to be present in the sediment. In March 1945 a diagnosis of tuberculous spondylitis of the 11th and 12th dorsal vertebrae was made and at this time acid fast bacilli were found in the gastric contents. In August 1945 a bilateral chorioretinitis was discovered, the eyegrounds having been negative in March. At the present time this boy is in fair condition awaiting surgical fusion of his spine. X-rays of his lungs have shown an entirely clear parenchyma since October 1945 (Fig. 6-B) and there is evidence of calcification in the spleen (Fig. 6-C).

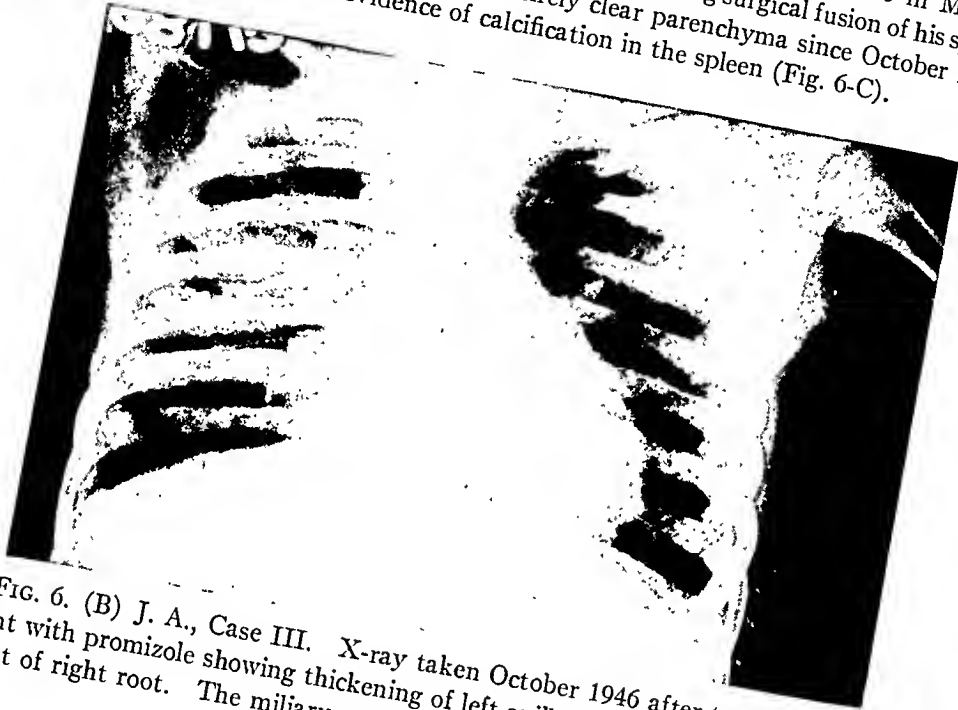


FIG. 6. (B) J. A., Case III. X-ray taken October 1946 after two years treatment with promizole showing thickening of left axillary pleura and slight enlargement of right root. The miliary mottling has completely disappeared.

Encouraged by our results in acute miliary tuberculosis we are now using promizole in other forms of tuberculosis of hematogenous origin. It is too early to report fully on our results, but we should like to report the result of treatment of one individual with chronic hematogenous tuberculosis who developed pulmonary miliary tuberculosis. The prognosis of this form of hematogenous tuberculosis is apparently not as serious as that of acute generalized miliary tuberculosis (Miller, 11).

Case IV. F. S. was first seen because of obvious hematogenous tuberculosis. In April 1944 at the age of eleven following an operation for a supposed thyroglossal cyst which proved to be a tuberculous lymph node, she developed dactylitis of hand and feet and in July 1944 a left sided pleurisy with effusion. Clouding of

both apices appeared in March 1945 and a pleurisy with a small effusion on the right in December 1945. Mottling of miliary size was first definitely seen in March 1946. In April a choroiditis was first noted. By June the mottling in the

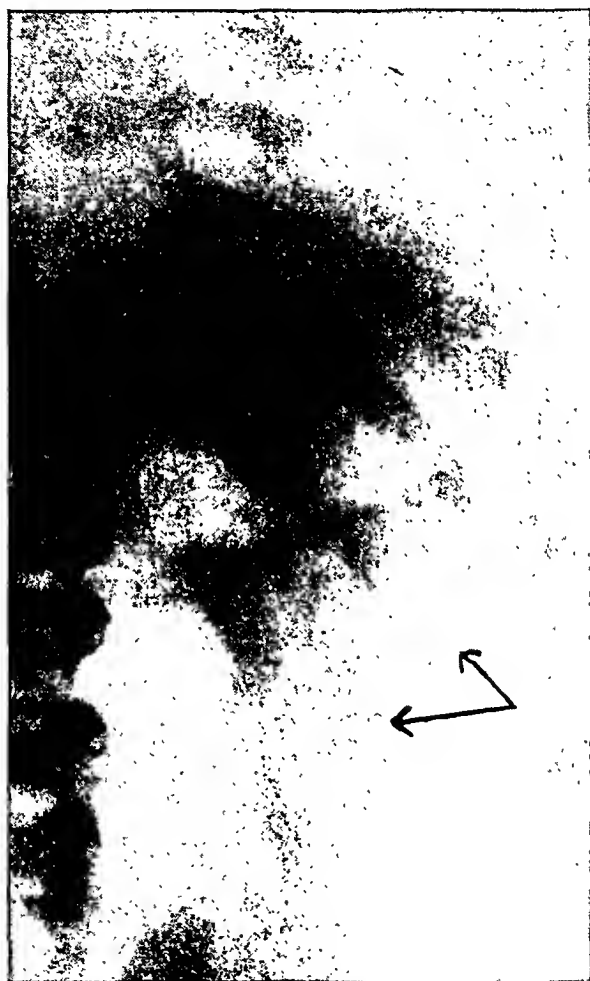


FIG. 6. (C) J. A., Case III. X-ray taken in 1946 showing calcifications within enlarged spleen.

lung fields had become very marked (Fig. 7-A). Physical examination at this time revealed a poorly nourished girl with dullness and diminished breath sounds at left base posteriorly. For the first time moist post tussic rales were heard over both apices. Liver and spleen were not palpable, there was enlargement of superficial lymph nodes. Promizole was started on June 22, 1946 and the dose increased to 4 gms. daily. The patient continued to receive this amount of the drug with

no evidence of toxicity. Shortly after treatment was begun tuberculosis of the right sacro iliac joint was diagnosed. There has been marked general improvement including a weight gain of $7\frac{1}{2}$ lbs. X-ray seven weeks after treatment was

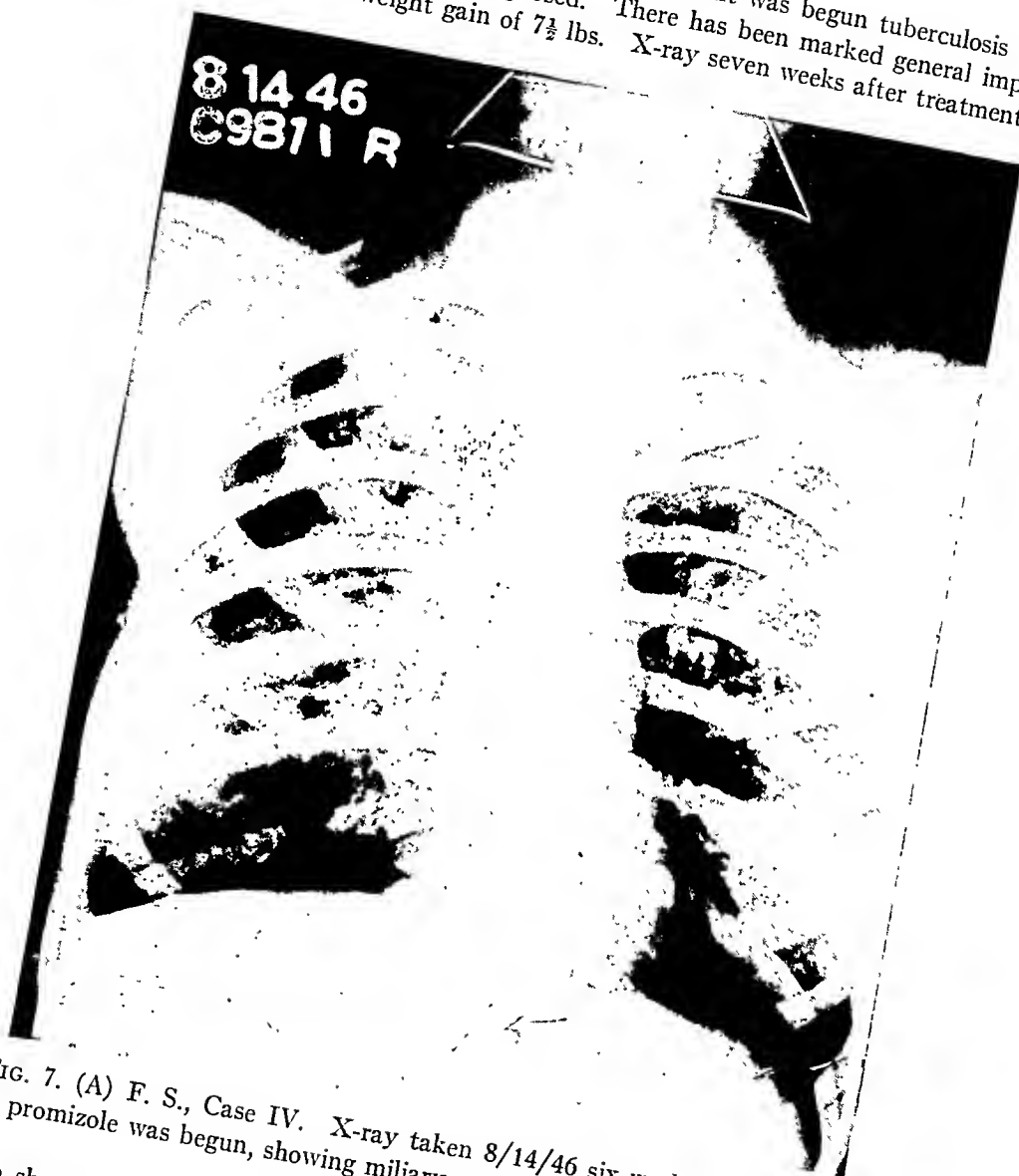


FIG. 7. (A) F. S., Case IV. X-ray taken 8/14/46 six weeks after treatment with promizole was begun, showing miliary mottling in both lung fields. begun shows no change but by December 23, 1946, (Fig. 7-B) six months after promizole was first begun, there is marked roentgen evidence of clearing and on March 28, 1947 no roentgen evidence of miliary tuberculosis can be found (Fig. 7-C).

DISCUSSION

All the evidence we have gathered tends to show that promizole acts slowly in the child and that its effect on tuberculosis of hematogen-

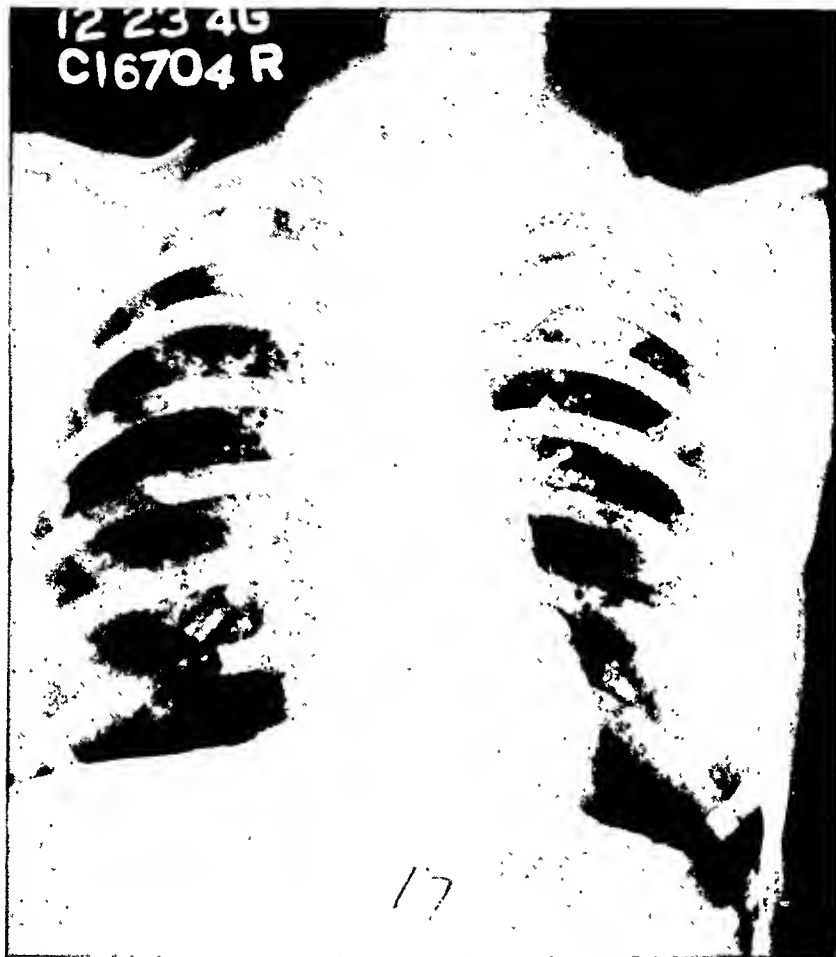


FIG. 7. (B) F. S., Case IV. X-ray after six months of promizole therapy (Dec. 1946) showing marked diminution in extent of miliary tuberculosis.

ous origin is slower than the results reported of antibiotics such as streptomycin. In the four cases where we have roentgen evidence of complete clearing of miliary tuberculosis, the mottling did not begin to diminish until ten to eighteen weeks after promizole was first ad-

ministered. We have earlier proof of bodily response to the drug in evidence of its goitrogenic action. While enlargement of the thyroid is usually first observed two to five months after promizole is first

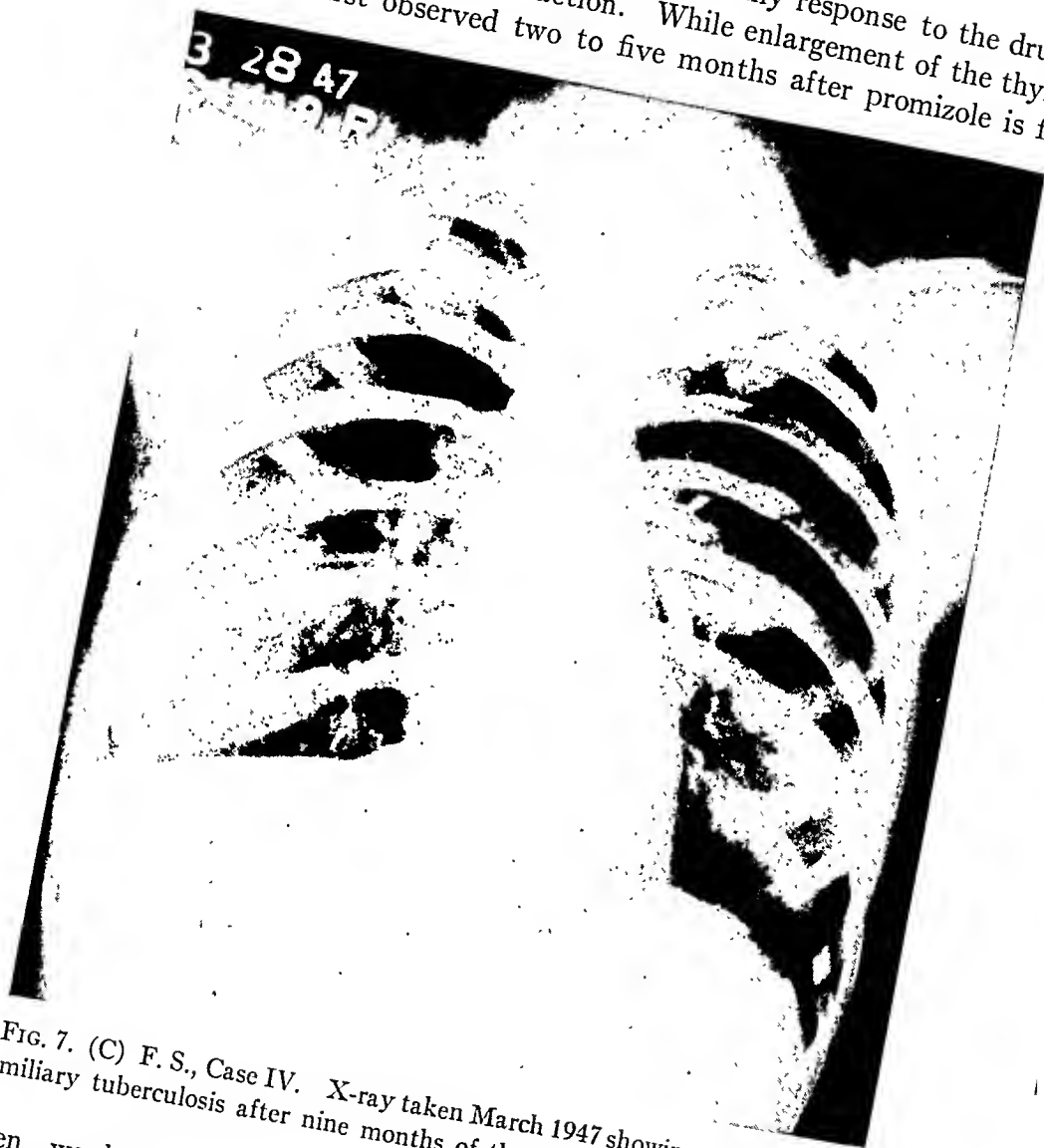


FIG. 7. (C) F. S., Case IV. X-ray taken March 1947 showing complete clearing of miliary tuberculosis after nine months of therapy.

given, we have pathological evidence of the goitrogenic action of promizole in the case who died 43 days after beginning treatment (Fig. 1).

Since most individuals with acute generalized miliary tuberculosis die within three months after first roentgen evidence of the disease, it

is obvious that we can not hope to arrest all cases of miliary tuberculosis with promizole. Chronic miliary tuberculosis lasting more than three months apparently has a better prognosis than the acute forms. Hoyle and Veazey (12) reported that 25% of individuals, usually adolescents or young adults, with chronic miliary tuberculosis go on to arrest of their disease. VanCreveld and Huet (13) reported 13 cases of recovery from chronic miliary tuberculosis during an observation period of 16 years. While we have never seen spontaneous recovery from miliary tuberculosis in children even where the disease became chronic both Engel and Wallgren have reported instances of recovery. We may speculate that an individual with miliary tuberculosis who survives the usual duration of three months shows more natural resistance.

The first five cases of acute generalized miliary tuberculosis treated for more than a month were consecutive cases. It is suggestive that the two cases who died were diagnosed only three weeks and one month before treatment was begun, whereas two of the three cases who have ultimately shown complete roentgen clearing had been diagnosed $3\frac{1}{2}$ to $4\frac{1}{2}$ months before treatment was initiated.

Our group of cases is small but in view of our past experience with miliary tuberculosis we feel that the evidence is striking that promizole may have a favorable action in suitable cases of miliary tuberculosis. In our next group of cases we plan to combine promizole with streptomycin in the treatment of miliary tuberculosis. Thus we hope to avail ourselves temporarily of the rapid action of streptomycin and then continue the treatment with promizole which has the great advantage of ease of administration, relative lack of toxicity and which can apparently be given safely and effectively over a period of years.

SUMMARY

Promizole has been used in the treatment of miliary tuberculosis in children. Five consecutive cases of acute generalized miliary tuberculosis were apparently adequately treated with promizole.

Of these, two children died 43 days and $4\frac{1}{2}$ months after therapy was begun.

Three children showed complete recession of roentgen evidence of miliary tuberculosis and are alive 30 to 33 months after promizole was

first given. In these cases who survived acute miliary tuberculosis the primary pulmonary lesions cleared slowly and in two cases went on to cavitation. In one case roentgen evidence of the primary focus is still present. One child developed tuberculosis of the spine, chondritis and positive urine cultures for tubercle bacilli while taking the drug. All three cases are still receiving maintenance doses of promizole.

One adolescent girl is reported who developed acute miliary pulmonary tuberculosis in the course of a chronic protracted hematogenous tuberculosis. The miliary lesions have disappeared while under treatment with promizole.

Promizole is goitrogenic and has a stimulating effect on secondary sex characteristics, causing enlargement of nipples and breast tissue and growth of pubic hair. No toxic effects were observed which would require permanent discontinuance of the drug. No irreversible toxic effects have been observed with the possible exception of the effect on sex maturation.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Cambridge Medical History. By SIR WALTER LANGDON-BROWN. 119 pp. Cambridge University Press, New York, New York, 1947.
In this delightful little book, Sir Walter Langdon-Brown describes the history of medicine in Cambridge in a series of seven biographical essays. These portray the work and personalities of such men as Caius, Gilbert, Glisson, Heberden, Hartley, Haviland, Paget, Humphry, Foster, and Allbutt. The book is well written and a worthy addition to items on medical history. It will interest all who have studied at Cambridge, as well as others interested in the particular personalities portrayed.

H. N. H.

Clinical Radiology. A Correlation of Clinical and Roentgenological Findings. Edited by GEORGE UTLEY PILLMORE. 2 vols. Illus. 1600 pp. \$45.00. F. A. Davis Co., Philadelphia, Pennsylvania, 1946.

This two volume work written by fifty-eight contributors has attempted to present the manifold phases of diagnostic roentgenology and their application to clinical problems. The manuscript has been divided into ten parts and includes sections discussing the cardiovascular system, the respiratory system, the gastrointestinal system, the urogenital system and the osseous system. Sections are also devoted to the brain, spinal cord and nasal accessory sinuses. Small chapters are also given to a consideration of soft tissue roentgenology and to the localizing of foreign bodies by roentgenographic methods.

Although there are several minor omissions, this work is unusually complete and brings together much of the fundamental knowledge in the field of diagnostic roentgenology. Like any work that is written by a number of authors, some sections of the manuscript are noticeably better than others. Sections devoted to cardiovascular disease and to intracranial lesions are particularly well done, and, in the case of the former, reflect the wide experience of the section's author in some of the more recent technical methods that have been developed. The section on the respiratory system is disproportionately large, and, as a result, some of the material is redundant. For example, in the discussion of pulmonary tuberculosis, the author presents several classifications of the disease which the average reader will find of very little use. Also, in this section, no reference is made to the place of histoplasmosis in the differential diagnosis of pulmonary calcification. Other omissions in this book are evident principally in the bibliographies which appear at the end of each section. Practically no reference is made to work performed outside of the United States; also, in this country, no reference has been made in the section on the gastro-intestinal system to the classical writings of Templeton.

In general, the format of the book is good. The printing is clear and the manuscript is well bound. The roentgenographic reproductions also are somewhat better than average, although, in a number of instances, the reproductions do not present the opacities in the same relationship as they were in the original films. However, in spite of its shortcomings, this work should be found valuable by students of diagnostic roentgenology and, in particular, by physicians who wish to have at hand a generally complete treatise on the various phases of diagnostic roentgenology.

R. H. M.

Curare, Its History, Nature, and Clinical Use. By A. R. MCINTYRE. Illus. 240 pp. \$5.00. The University of Chicago Press, Chicago, Illinois, 1947.

This monograph, by an author long active in investigation of the properties of curare, furnishes a most useful compilation of scattered information which has been gathered since Claude Bernard first described accurately the action of curare. The first chapters are devoted to a scholarly review of accounts of early explorers of the South American continent, regarding observations on the sources, preparation, and use of curare by the native Indians. Then there is a full section on the botanical characteristics of the various curariform plants. The remainder is devoted to the functional inter-relationships of curare, curarising and decurarising agents, and the host of substances which affect neuromuscular function. This section of the monograph cites almost all of the observations of many investigators; however, no clear principles of function result. Perhaps, owing to the fragmentary nature of our knowledge of underlying mechanisms in this field, no clearer analysis is possible at this time.

J. L. L., Jr.

Dynamic Aspects of Biochemistry. By ERNEST BALDWIN. Illus. 457 pp. 21 s. net. University Press, Cambridge, England, 1947.

Here is the type of biochemistry book of which more are needed. Many scientific workers in various fields seek an exposition of biochemistry which does not emphasize the more or less straight-forward, general, textbook background on the one hand, nor delves deeply and exclusively into one narrow field on the other. Rather there is wanted a broad discussion of the more fluid and detailed aspects of a number of biochemical subjects integrated into the outlines of biology as a whole. This book is a big contribution in this direction.

Under the general heading of "Enzymes", there are taken up as successive chapters, "The General Behaviour and Properties of Enzymes", "The Nature of the Catalytic Process", "Hydrolases and Phosphorylases", "Oxidizing Enzymes", and "Other Enzymes". Under the general heading of "Metabolism", the author discusses, "Methods Employed in the Investigation of Intermediary Metabolism", "Food, Digestion and Absorption", "General Metabolism of Proteins and Amino-Acids", "Special Metabolism of the Amino-Acids", "Excretory Metabolism of Proteins and Amino-Acids", "Some Special Aspects of Nitrogen Metabolism",

BOOK REVIEWS

"Metabolism of Purine Derivatives", "Anaerobic Metabolism of Carbohydrates, Alcoholic Fermentation", "Anaerobic Metabolism of Carbohydrates, Muscle and Liver", "Aerobic Metabolism of Carbohydrates", and "Metabolism of Fats". The book is well integrated and written in an easy, conversational style. Historical background is woven into the general discussion at the appropriate place, not taken up as a separate section. The same is true of the more usual factual background which is brought in.

Attention is paid to the development of our concepts of biochemical physiology and not just to fact. Some of the thoughts developed by the author are quite challenging, such as: "In view of these considerations, we must inquire whether we have perhaps been led astray by studying muscle metabolism only under anaerobic conditions, and whether anaerobic contraction has any real biological significance at all." In the discussion of purine metabolism, little is said about more recent knowledge of sources of carbon and nitrogen in these substances. The book is to be highly recommended and can be read through to advantage and with sustained interest. It is also an excellent reference work on general topics. It is to be hoped that more books of this type will appear.

F. W. B., JR.

Experiences with Folic Acid. By TOM D. SPIES. Illus. 110 pp. \$3.75. The Year Book Publishers, Inc., Chicago, Illinois, 1947.

The author's remarks are based on a most extensive clinical experience with folic acid therapy. Observations are reported on 218 cases. Some of the hematological responses among cases of Addisonian pernicious anemia, tropical sprue, nutritional macrocytic anemia, and cirrhosis of the liver were dramatic. The last group of patients is most worthy of note. There are presented the basic structural formulae of compounds with which clinical and laboratory investigations are being made. This is a book worthy of an evening's perusal. However, it does not emphasize that present clinical experience indicates folic acid therapy in pernicious anemia, in our present state of knowledge, may be very dangerous. Severe, sudden, and frequent neurological relapses occur in an unpredictable manner.

P. W.

Operative Gynecology. By RICHARD W. TELINDE. Illus. 751 pp. \$18.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1946.

Doctor TeLinde's book on operative gynecology is a valuable addition to gynecological literature. It consists of 751 pages and covers the whole field of the surgical treatment of gynecological disorders. The subject matter is divided into thirty-eight chapters and is well presented. There are 324 illustrations and an excellent index.

Because of its completeness and the excellence of its illustrations, this volume cannot help being useful to practicing surgeons as well as to internes and house officers. The author stresses not only operative technic and surgical principles,

but also the role of nonoperative therapy in many gynecological disorders. The indications and methods of irradiation therapy are also well presented. In general, therefore, after reading this book carefully from cover to cover, this reviewer closes it with appreciation of its excellence and feels certain that it will find wide acceptance.

L. R. W.

Parenteral Alimentation in Surgery, By ROBERT ELMAN. 267 pp. \$4.50. Paul B. Hoeber, Inc., New York, New York, 1947.

This volume, which represents a full length presentation of the entire problem, was originally awarded the quinquennial Samuel D. Gross prize of the Philadelphia Academy of Surgery. It is a monograph of 267 pages carefully and simply written, carrying tables, figures, and references which serve to amplify well the text. While much of the basic material is known to all who have kept abreast of advances in parenteral therapy, the reader is easily led into the more technical aspects as each chapter unfolds its own special subject matter. A consideration of water and electrolyte needs, of caloric needs, and vitamin needs precedes the more-emphasized consideration of protein needs. The author, a well recognized authority, then discusses plasma and whole blood transfusions, pure amino-acids vs. hydrolyzed protein and the many related problems of their preparation and use. Finally, a practical program for parenteral alimentation is offered. Dealing effectively and simply with one of the frontiers of clinical surgery, this volume represents a source of well compiled information to which clinicians and investigators should frequently turn.

S. McL.

STUDIES ON THE PHYSIOLOGY, BIOCHEMISTRY, AND CYTOPATHOLOGY OF THE CORNEA IN RELATION TO INJURY BY MUSTARD GAS AND ALLIED TOXIC AGENTS

BY MEMBERS OF THE STAFF OF THE WILMER INSTITUTE

I. INTRODUCTION AND OUTLINE

JONAS S. FRIEDENWALD AND ALAN C. WOODS.

A study of the ocular injuries produced by chemical agents was begun at the Wilmer Institute in the summer of 1941. A few months later the Committee on the Treatment of Gas Casualties of the National Research Council requested that this work be greatly expanded and accelerated. A contract was negotiated between the Office of Scientific Research and Development and the Johns Hopkins University for the support of the expanded program in November 1941, and this support was continued until the end of 1945. The story of the arrangements under which work in this and related fields was carried out in a large number of laboratories has been told by Dr. Milton C. Winternitz (1), who acted as Chairman of the Committee on the Treatment of Gas Casualties and whose wise judgment and driving enthusiasm gave form and flexibility to the whole project.

In the first World War the incidence of casualties due to the ocular effects of mustard gas was extremely high, some ninety per cent of all gas casualties having ocular symptoms. For the most part these were relatively mild vapor burns which left no permanent damage to the eyes, but which prevented the individual from performing active duty for a few days to several weeks. Throughout the first part of World War II competent military authorities felt that there was considerable probability that gas warfare would be resorted to, and it was not until after the successful landing in Normandy that anxiety in this matter diminished. Research in this field was, therefore, under the pressure of imminent concern.

During World War I, research on the ocular injuries produced by war gases was largely limited to clinical and histopathological studies

on man and animals. Some laboratory experiments were performed seeking to establish a definitive treatment with various irrigations, ointments, etc., all of which were, however, found to be essentially inefficacious. In the work undertaken in the Wilmer Institute, it was realized from the outset that the chances were very small indeed of finding an effective treatment of mustard injuries of the eye by a continuation and expansion of the empirical methods of study used during World War I. Consequently, a program of study was developed on the broadest feasible base in the hope that, if we knew more about the mechanisms by which tissue damage results from toxic injury, some therapeutic possibilities might be disclosed. It must be admitted at once that no cure for mustard injury was discovered. However, the search for the mechanism of mustard injury led us to study many aspects of normal corneal physiology that had not been previously explored. These phases of our study are reported in the series of papers which follow. Some parts of the investigation in respect to toxic agents other than mustard have been reported elsewhere (2-7).

OUTLINE OF THE INVESTIGATION

I. *Preliminary and Exploratory Studies.* As a first step it seemed desirable to discover whether there were any recognizable distinctions in the severity and type of clinical and histological reactions of the cornea to a variety of chemical agents of known reactivity. These studies were conducted by Dr. Wm. F. Hughes, Jr. It soon became apparent that some substances were well tolerated by the cornea when dropped on its surface but caused severe damage when injected into the tissue. Some useful information as to the permeability of the tissue may be gathered from this type of comparison. In order to study the intrinsic toxicity independent of penetrating power, we employed intracorneal injections in most of our tests. The results of this study are compiled in Appendix I.

It is possible to divide the injurious agents into two major groups, those that are damaging only in massive concentration and those that are damaging in very small doses. Among the former are acids and alkalis which cause injury by pH change or protein precipitation, hygroscopic organic solvents such as propylene glycol, hypertonic

solutions of inorganic salts, etc. Among the second group that are damaging in low concentrations are heavy metal ions, oxidizing agents including quinones, and alkylating agents. A possible common point of attack of this latter group are the sulfhydryl groups of the tissue. A ketobinder such as semicarbazide was tolerated by the tissues in considerably higher concentration than were the sulfhydryl attackers. The same is true of agents such as KCNO and formalin which react with amino groups.

While these results suggest that the war gases capable of damaging the cornea in very small doses may operate through an attack on the tissue sulfhydryl groups, the implication is by no means strong. In one sense these experiments were disappointing. No general distinctions could be found either in respect to the clinical or histological course of the lesion which could be related to the postulated mode of chemical attack of the injurious agent. Individual agents exhibited minor differences in the spreading of their effect, or the intensity of the purulent reaction which they elicited, but there were no systematic differences found between the effects of sulfhydryl binders such as arsenite, ketobinders such as semicarbazide, and amine binders such as formalin, or even between these groups and such unique agents as fluoride, quinine, cyanide, and the like. Even the effect of beta radiation, though remarkable for its long incubation period, is not grossly distinguishable from that of the various chemical agents. It is to be concluded that the clinically and histologically recognizable changes which follow chemical damage of the tissue are only remotely and indirectly connected with the initial chemical injury. Consequently, observations on these remotely connected events throw no light on the nature of the initial chemical injury.

This was perhaps to have been anticipated, for the clinical and histological reactions disclosed by routine study are, in the main, the consequences of cellular death. Once the cells are dead the reactions have a monotonous uniformity. This does not preclude the possibility that a study of the mode of death would be equally unilluminating, but such a study was beyond the scope of the routine examinations that were pursued in this phase of our investigation.

In spite of this disappointment these studies were not without value. They gave us a broadly based knowledge of the general course of

clinical reactions to tissue damage in the eye upon which we were able to construct a numerical index of the severity of the injury, an index which was to prove useful in the evaluation of therapeutic agents in later work. The method of applying this numerical index has been reported elsewhere (2). Moreover, knowledge was gained of a variety of agents that are surprisingly well tolerated by the ocular tissues. This knowledge was useful in the subsequent choice of possible therapeutic agents and vehicles. This aspect of the work was on a rather limited scale and could profitably be greatly expanded. Finally, a comparison of the effects of surface application with those of intracorneal injection furnishes a useful tool for the study of the permeability of the surface barrier, a subject which merits further extensive study.

II. Clinical and Histological Studies on the Ocular Effects of Various War Gases. Following these preliminary exploratory studies much more extensive and detailed studies of a similar kind were made by Drs. Hughes, Scholz, and Maumenee, using various war gases: mustard, the nitrogen mustards, lewisite, and one of the fluorophosphates. The studies by Hughes on lewisite have been reported elsewhere (5). Those by Maumenee and Scholz on mustard and the nitrogen mustards are presented in one of the succeeding papers (see page 121). Much of our effort in these studies was directed toward the production of a standard and reproducible lesion of submaximal severity for subsequent therapeutic tests.

Special reference should be made to the study by Dr. Scholz of the effects of di-isopropyl fluorophosphate which was found to be well tolerated by the ocular tissues, producing no local permanent damage even when administered locally in barely sublethal dosage. The analysis of the intense cholinergic effects produced by this agent in the eye is reported elsewhere (6, 7).

III. Role of Secondary Infections. The influence of secondary infections on the course of ocular injuries with war gases was studied by Drs. Maumenee, Guyton, and Burky. Using fairly severe injuries it was found that the suppression of infections by penicillin and sulfonamides resulted in a slight but definite reduction in the severity of the ocular reaction. Comparing these results with those of investigators in other laboratories, it would appear that the severity of

the secondary infection varies widely from one laboratory to another, and that more striking benefit from antibacterial agents is obtainable if the initial injury by the war gases is relatively mild. It was concluded that the results of studies on laboratory animals did not afford a valid basis for predicting how important secondary infections might be in humans, but that under proper precautions the antibacterial agents could be administered without danger of increasing the injury.

IV. *Antidotes.* 1) BAL (British Anti-Lewisite). The remarkable achievement of the British investigators, Peters, Stocken, and Thompson (8) in the synthesis of this compound and the demonstration of its anti-arsenical action placed the whole subject of the immediate treatment of war gas injuries in a new and different perspective. When this material first became available in this country, our laboratory, among others, was requested to study it. The matter appeared at the time to be one of great urgency and was pushed with the maximum possible speed. Using rabbits as the experimental animals, Dr. Hughes (5), who undertook this study, was able to work out the dosage of BAL tolerated by the eye, the effect of previous administration of lewisite on this tolerance, the therapeutic ratio of BAL, the influence of the time interval between exposure to lewisite and the administration of the antidote, the relative efficacy of the antidote against vapor and splash burns, etc. An effective ointment base was devised in which the antidote could be incorporated.¹ These results were rapidly extended to experiments on monkeys and, in relation to the tolerance of the eye for BAL, on human volunteers, and contributed a substantial base for the subsequent working out of the BAL eye ointment by others.

2) The Search for Antidotes against Mustard. The possibility of obtaining a completely effective detoxifying agent for mustard.

¹This ointment base was devised by Mr. Fuqua, pharmacist of the Johns Hopkins Hospital. Owing to security restrictions, it was not possible to tell him the nature of the material with which he was asked to work nor the purpose for which the ointment was being prepared. Nevertheless, with rare intuitive skill, Mr. Fuqua developed an effective ointment. The ointment base which he prepared has been frequently erroneously referred to as the "Friedenwald base." The writer wishes to take this occasion for making it clear that he had nothing whatever to do with the preparation of this ointment.

is rather poor, because no substance has been found which will remove mustard from its combination with proteins without destroying the proteins. Consequently it can be hoped only that an antidote will neutralize the mustard which remains on the surface of the tissues plus that which has entered the tissues but has not yet reacted with them when the antidote is applied. It has been shown by others that in the skin approximately 10 minutes is required for a locally destructive dose of mustard to penetrate, while the half life of the mustard which has penetrated is of the order of two minutes. Consequently, within 5-10 minutes after exposure, surface decontamination is partially effective, but after 10 minutes almost all of the mustard which has penetrated has reacted with the tissues. In the eye the same argument holds a fortiori in respect to vapor burns, which in World War I produced most of the casualties. Consideration that mustard might be sprayed from airplanes during World War II enhanced the dangers of droplet contamination and made it desirable to explore the possibility of developing a penetrating decontaminant for the ocular tissues.

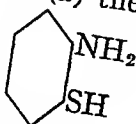
It was found that mustard penetrates the cornea more rapidly than the skin so that the time after exposure in which surface cleansing is still partially effective is very short (2-3 minutes). The rate of reaction of penetrated mustard with the cornea is of the same order of magnitude as its rate of reaction in the skin, the only favorable factor being that the cornea, because of the constant evaporation of the tears, is somewhat cooler than the skin. There is, consequently, a brief interval between 2 and 5 minutes after exposure during which a penetrating decontaminant might be expected to be beneficial.

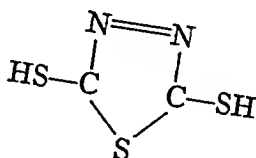
Within these limitations the agent to be sought for would be one with high reactivity for mustard, capable of penetrating into those regions in the tissues and in the cells reached by mustard, forming a non-toxic reaction product with mustard, and is itself non-toxic in therapeutically effective dosage. The problem is further complicated by the fact that the anterior corneal surface presents a relatively impermeable barrier of rather special character. It seemed wiser to avoid this special problem at the outset and to discover whether or not there were any substances which, if injected into the tissue simultaneously with mustard, would prevent its damaging effect.

To this end a screening test was devised in which mustard and the potential antidote were mixed in aqueous solution and immediately injected intradermally into rabbits. The same mixture was allowed to stand at room temperature for half an hour and then injected. Controls using mustard alone and the antidote alone were also injected. Initially lecithin was introduced into the injected mixture in the hope of imitating in this way the biphasic aqueous-lipoid arrangement of the tissues. Control experiments without lecithin showed that presence of this substance did not alter the test. Substances which effectively protected the tissue against mustard in the presence of lecithin also did so in its absence. Substances which were ineffective with lecithin were ineffective without it. Nevertheless, the presence of lecithin in the mixture was of considerable practical advantage, for the mustard in lecithin aqueous emulsion maintains its potency much longer than in the absence of lecithin. Consequently, the time interval between preparing the mixture and its injection is less critical when lecithin is included. Substances insoluble or poorly soluble in water were dissolved when possible in ethyl alcohol and then introduced into the lecithin mustard water emulsion in such quantity that the total concentration of alcohol was non-irritating. Later, dimethyl formamide was found to be a satisfactory vehicle for some substances insoluble in both water and alcohol. Substances insoluble in water or ethyl alcohol were dissolved when possible in dimethyl formamide and then introduced into the lecithin mustard water emulsion in such quantity that the final concentration of dimethyl formamide was non-irritating.

Several hundred substances of many chemical types were tested in this manner.² Three groups were found which contained some

² Some of these materials were available commercially. Others were supplied by Dr. Hellerman of the Department of Physiological Chemistry of the Johns Hopkins Medical School. A considerable number of biochemical substances were contributed by Merck and Co. Dr. Bergmann of the Rockefeller Institute contributed some materials especially prepared as possible antidotes for mustard. By far, the most important contribution of substances was supplied by the Chemical Department, Experimental Station of the duPont de Nemours Co., under a contract arranged by the 9-1 Division of the NDRC for this purpose. The cooperation and suggestions of Dr. Lazier and later of Dr. Howk who conducted the synthetic work at the duPont Laboratories, and of Dr. Marvel and later of Dr. Adkins as chairman of the 9-1 Committee are deeply appreciated by us.

effective members: (a) the dithio-carbamates $R_2NCSSNa$, (b) ortho-amino-thiophenol  and certain of its derivative and analogues, (c) 2,5 dimercapto-thio-diazole.

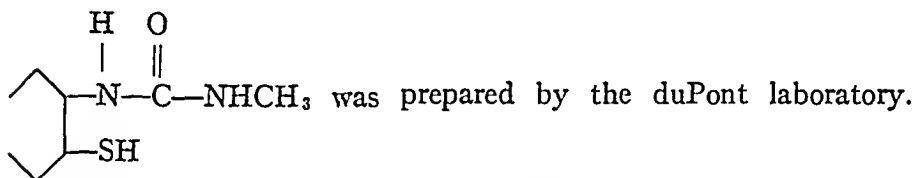


(a) A considerable number of dithiocarbamates were tested on rabbits' eyes following droplet contamination with mustard and nitrogen mustard. The lower members of the series, especially diethyl and diethanol dithiocarbamates proved the most effective and, under optimal conditions achieved close to the theoretically maximum therapeutic effect. Several disadvantages were found which made these substances unsuitable for supply to combat troops. They were found to be effective only when administered in concentrated aqueous solutions and the problem of packaging such solutions presented enormous difficulties. Salves and jellies were not found effective. Owing to the high hypertonicity of the effective solutions they caused marked immediate blepharospasm and lachrymation when administered to an unanaesthetized eye and achieved their maximal therapeutic benefit only when administered under general anaesthesia, consequently self treatment with them would be very difficult if not impossible. Finally, these substances could not be combined with BAL in the same preparation because of their mutually exclusive pH stability zones. It was not thought feasible to supply combat troops with two separate antiwar-gas eye preparations, nor to abandon BAL, which itself has some protective value against mustard in the eye, for another preparation which was only slightly better against mustard and had no value against arsenical agents. Nevertheless, the possible use of dithiocarbamate solutions for first aid in mustard factories was recommended.

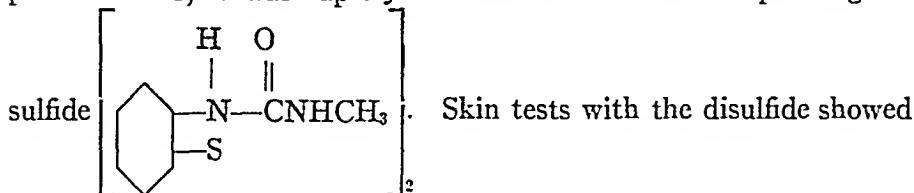
(b) Ortho-amino-thiophenol was the most effective substance found in the screening tests, in terms of the minimal molar ratio of

antidote to mustard that was protective. This substance is, however, quite toxic and its therapeutic index (minimal effective dose divided by minimal toxic dose) was very small. No mode of administering this substance to the eye effective after droplet contamination with mustard was found. A derivative of this compound, 3 mercapto 4 amino benzoic acid, and also its ethyl ester were found capable of some therapeutic benefit. These are, however, quite toxic and could be used only in low concentrations. Oxidation of these compounds and subsequent reduction by sulfhydryl containing substances resulted in an increase in their toxicity. It seemed possible that in such an experiment derivatives of the original compound were being formed which had an increased tendency toward quinonoid oxidation and that a similar course might explain the high toxicity of the original material in the tissue. Following the line of argument plans were formulated for the study of various N substituted derivatives of ortho-amino thiophenol.

One such compound (ortho-mercapto-phenyl-methyl-urea)



It proved to be so intensely auto-oxidizable that, in solution on exposure to air, it was rapidly converted into the corresponding di-



it to be relatively non-toxic. Solutions of the disulfide were reduced by H_2S and used with mustard in lecithin emulsion in the screening skin test. The compound was found to be about as effective against mustard as was 3 mercapto 4 amino-benzoic acid and also about as toxic as this previously tested substance. The nature of this toxicity was not further explored.


(c) The compound 2,5 dimercapto-thiodiazole was itself extremely

toxic and was not further explored since no possibility suggested itself as to how non-toxic derivatives might be produced.

We regret very much being compelled to abandon the search for an anti-mustard antidote with the job neither satisfactorily accomplished nor proven impossible of solution. An analysis of our data can yield only tentative conclusions but is offered here in case it should be found necessary to reopen this problem in the future. All of the compounds which were found effective in our screening test contain sulfhydryl groups whose acid dissociation is below pH 7. Consequently, they all presumably have quite high competition factors for mustard. But there are many other substances with equal or higher competition factors, for instance thiophosphates, which were found ineffective on the screening test. The presence of a nitrogen atom in close proximity to the SH group seems necessary for their effect. The xanthates $R-O-C(=S)SH$ and the monosubstituted tri-

thiocarbamates $R-S-C(=S)SH$ which are chemically closely related

to the dithiocarbamates proved ineffective. The same is true of thiophenol and a number of its derivatives which lack the orthoamino

group. On the other hand alpha-mercapto-pyridine  was

slightly protective. When we first noted this relationship we suggested that the effective compounds might derive their efficacy from their capacity to form internal salts, but a subsequent study of the dissociation constants of the potential basic groups revealed them to be such weak bases that they could furnish only infinitesimal concentrations of dipole-ions at physiologic pH. A possible alternative explanation is that these compounds may exist in part as chelate structures. Such uncharged molecular species might be able to

penetrate into intracellular regions inaccessible to the anions of simple sulfhydryl acids. No studies have been made by which this hypothesis could be tested.

V. Primary Site of Mustard Reaction. With threshold doses of mustard, the only change in the corneal tissue that we have been able to recognize is a transitory inhibition of mitotic activity in the corneal epithelium. It would be of interest, therefore, to know whether the primary site of reaction of mustard with the cell is in the cytoplasm or in the nucleus. The lipid solubility of mustard and its unreactive nature in the absence of water suggests that the aqueous phase of the tissues immediately adjacent to lipoidal structures might be the site of greatest reactivity of mustard:

(1) The electrical resistance of the cornea is not immediately affected by exposure to mustard. Some hours after exposure the resistance declines moderately (about 25%), and when the epithelium sloughs off it declines to zero. It may be concluded that the lipoidal phase of the cell membrane is not disrupted as the immediate result of the reaction of mustard with the tissue (see page 102).

(2) The nuclei of the corneal stroma cells show increased fragility after exposure to mustard but this is not an immediate result of exposure to mustard (see page 161).

(3) None the less it is evident that the corneal epithelial cells do not behave toward mustard as if they were homogeneous solutions. These cells in the beef eye contain considerable amounts of glutathione. If the corneal epithelium is scraped off from beef eyes, suspended in a small volume of salt solution and an amount of mustard added equimolar to the content of glutathione, no measurable decrease in the titratable glutathione is observed (experimental error 5%). If, however, a similar preparation of epithelial cells is first ground with broken glass and then the same amount of mustard added to the resulting emulsion, about 50% of the previously titratable glutathione disappears. It is evident that in the intact cell glutathione is relatively inaccessible to the mustard (see page 102).

(4) After very heavy exposures to mustard vapor there is an increased eosinophilic staining of the corneal tissues. This increased capacity to bind acid dyes is apparently due to the formation of some new basic groups in the tissue as the result of its reaction with mustard.

A possible explanation of this phenomenon is to be found in the tendency of mustard to form sulphonium compounds in certain reactions. After very heavy exposures the increased eosinophilia is uniformly distributed within the corneal epithelial cells, but as the dosage is reduced the regions about the nuclear membrane and the basement membrane appear to be the last to lose their enhanced eosinophilia. These results were obtained at the very limit of the sensitivity of the method used to observe them and are open to grave question. Nevertheless, they suggest that two of the regions specially susceptible to mustard attack may be in or adjacent to the nuclear membrane and the basement membrane (see page 102).

VI. *Nuclear Changes Produced by Mustard.* In the search for a field of research which might diminish the enormous gap in our knowledge between biochemical studies and those of clinical and histological pathology, we have investigated some of the nuclear disturbances which follow exposure to mustard. These studies were made by Dr. Friedenwald with the aid of Dr. Buschke.³ The results of these studies are reported in detail in separate articles below (see pages 148, 161, 178).

(1) *Inhibition of Mitosis.* Inhibition of mitosis occurs after exposure to very small doses of mustard and allied agents, and represents the most sensitive index, so far discovered, of threshold effects produced by these agents. The inhibition comes on slowly and can be made to last for as long as a week without being associated with

³ Friedenwald and Buschke had been engaged for some time in the study of mitotic and wound healing activities in the corneal epithelium. When it was decided to apply to the mustard problem the technique gained in this field arrangements were made to apply for the clearance of Dr. Buschke so that he could become a member of the group under OSRD contract. All concerned approved this step, but when application for clearance had been prepared it was learned that Dr. Buschke's father and mother had been taken into custody by the German government and placed in a concentration camp. Since the lists of those engaged in scientific work for the OSRD might well be available to German espionage it was decided that the inclusion of Dr. Buschke in our project might place unnecessary risks upon the lives of his parents. Consequently, Dr. Buschke agreed to work as a voluntary assistant on this problem, and assisted in the studies with great devotion in spite of being excluded from all knowledge of the nature of the agents used in the experiments and of all other confidential and secret information. His contribution to the work has been of very great value.

any signs of irritation or tissue destruction. No evidence is available with which to decide whether the primary damage responsible for mitosis inhibition is due to a reaction of mustard with nuclear or cytoplasmic components. The healing of small wounds by migratory action of the cells is not disturbed during the period of mitosis inhibition. Recovery from the inhibition is associated with a full return to normal. The inhibition occurs at levels of dosage insufficient to produce any of the metabolic disturbances which we have so far discovered.

(2) Nuclear Fragmentation. With doses of mustard somewhat greater than the minimum required to inhibit mitosis, but at thresholds still below the level of producing necrotizing lesions, some cells in the basal layer of the corneal epithelium show a form of karyorrhexis which we have called nuclear fragmentation. This peculiar mode of cellular death has been subjected to extensive study.

Under ordinary experimental conditions only a small number of the cells in the basal layer of the corneal epithelium are susceptible to this type of injury, but after various pretreatments the number of susceptible cells can be greatly increased. It is concluded that susceptibility to this type of injury is associated with some particular physiologic state of these cells though there are other tissues in which the cells are either uniformly susceptible or uniformly refractory to this type of injury. Evidence has been obtained which suggests that the susceptible state in the corneal epithelium is connected with the mitotic cycle, and that it may provisionally be identified as the pre-mitotic state, while the process of nuclear fragmentation itself may be provisionally identified as a pathological and incomplete form of mitosis. It thus appears that mustard and related substances can furnish important experimental tools for the study of mitosis. In this respect they deserve to take a place along with ultraviolet light and X-ray. Severe nuclear fragmentation, like mitosis inhibition, occurs at a dosage level which still fails to produce the metabolic disturbance noted below.

(3) Nuclear Fragility. The mode of death of the cells of the corneal stroma after exposure to mustard differs radically from the mode of death of the epithelial cells. Nuclear fragmentation does not occur in the corneal corpuscles. On the contrary after moderately severe

exposures the nuclei of these cells first swell and then burst. A study of this phenomenon showed that the swelling of the nuclei of the corneal corpuscles was a consequence of the imbibition of water by the cornea following damage by mustard to the corneal endothelium, and that similar swelling of the nuclei of the corneal corpuscles occurs after many types of damage to the endothelium. However, in corneal edema produced by mechanical destruction of the endothelium, the nuclei though swollen do not burst. The specific effect of mustard on these cells is, therefore, an increase in the nuclear fragility. The increased fragility of the nuclei appears only after some hours of incubation following exposure to mustard. Following massive exposures, cells of all types undergo pycnosis.

The phenomena of nuclear fragmentation and nuclear fragility develop not only in vivo but also in enucleated eyes maintained supravitaly in an incubator. It is possible, therefore, to vary widely the experimental conditions both before and after exposure to the toxic agent. Experiments of this type have thrown some light on factors favorable and unfavorable to the accumulation of cells in the susceptible state and also on factors favorable and unfavorable to the development of the lesions. In the past, studies of this sort have been made by others on tissue cultures or on monocellular organisms. The cornea appears to be an extremely suitable organ for extension of the techniques of cytopathology from the field of single cells into that of organized structures. A further extension in the same direction is exemplified in the section which follows.

VII. *Cohesion of the Corneal Tissues.* One of the early pathological results of mustard injury is a loosening of the corneal epithelium. This can be seen both in the intact animal and in the isolated incubated cornea. Studies on this phenomenon were undertaken by Dr. Herrmann and are reported in detail below (see pages 182, 208, 213). The cohesive surface appears to be a protein-lipoid multilayer since it is disrupted both by trypsin and by such substances as butyl and amyl alcohol and some other detergents. Energy may be necessary for the normal maintenance of cohesion, for while cyanide and anoxia do not cause a loosening of the tissue, iodoacetate and fluoride do cause such a loosening. Histamine and also freezing of the tissue with subsequent incubation also lead to a loosening of the epithelium. It

is likely that this phenomenon is closely related to that of vesication in the skin.

Exposure to mustard causes no immediate loss of cohesion, but on subsequent incubation the epithelium becomes loosened. The loosening of the epithelium is not a consequence of the death of the epithelial cells, for small wounds in the epithelium heal normally even when the tissue has become loosened. The loosening of the epithelium does not occur on anaerobic incubation, and some recovery from this toxic effect of mustard is achieved by the tissue during anaerobiosis. It is evident that the pathologic process caused by mustard which leads to the loosening entails an oxidative step. Reduced temperature also slows down or prevents the development of the loosening, and some recovery from the injury takes place during incubation at the lower temperature.

Nuclear fragmentation is also inhibited by anoxia and by lowered temperature, but the temperature coefficients of the two pathological processes are not identical. Under proper experimental conditions either loosening or nuclear fragmentation can be produced alone. This rules out the possibility that the loosening is the result of the accumulation in the tissue of toxic products derived from the autolysis of cells that are undergoing nuclear fragmentation.

VIII. *Studies on Corneal Metabolism.* Work in this field was conducted by Dr. Herrmann. In principle there are three modes of studying the effect of a poison on tissue metabolism. In the first place one may isolate various enzymes from the tissue and study their sensitivity to inactivation by the toxic agent. This mode of study has been widely pursued by others and has added much to our knowledge of the reaction of mustard with proteins in general, but has thrown little light on any possibly crucial enzymes upon the inhibition of which the pathological effects of mustard injury may be supposed to depend. We have not undertaken work in this field, since it was well covered by others. Furthermore there are some *a priori* objections to this mode of attack in relation to the specific objectives of our study. The known enzymes constitute only a fraction, possibly only a small fraction, of all those necessary for cellular activity. Even if an enzyme exquisitely sensitive to inhibition by mustard could be found the question would still remain open as to whether

this enzyme was accessible to mustard in the intact tissue (cf. the inaccessibility of glutathione to mustard in the corneal epithelium). Finally, it is possible that enzyme systems may be inactivated through structural changes or losses of intermediate factors without injury to the enzymes themselves.

A second mode of study, which has also been pursued by others, consists in exposing the tissues to mustard and then testing whether particular enzymes can be isolated in normal or diminished activities. This method has yielded some fruitful results in the hands of a number of investigators. It is free from the second objection mentioned above, but not from the other two objections. Furthermore, diminished extractability and diminished activity need not be identically related. Finally, work of this type was a natural corollary to that of the first type and was being actively covered by the efforts of others.

A third method of approach, the one we have followed, has been to study the utilization by the normal and poisoned tissue of various primary and intermediate metabolites. Once the interruption of particular metabolic activities could be demonstrated, the precise locus of the injury to enzymes, structure, or intermediate factor, could, we hoped, be attacked with more confidence. Work in this field was necessarily slow. At the outset almost nothing was known about the normal metabolism of the cornea, and only those metabolites could be tested for which satisfactory micro-estimation methods existed or could be elaborated. On the other hand, the cornea seemed an especially favorable tissue on which experiments of this type might be performed. Its avascular character renders its supravital maintenance extremely simple. Its anatomical simplicity made the separation of epithelium from connective tissue readily feasible, and hence made possible the study of the metabolic effect of poisoning on the separate tissue components.

The results of these studies are reported in detail below (see pages 225, 251, 260, 273, 287, 295). There is no evidence that the overall supply of available energy in the mustard poisoned tissue is defective because the conversion of glycogen to lactate proceeds at a normal rate. There is, however, some diminution of oxygen uptake. Moreover, as noted above, some abnormal utilization of oxidative energy occurs in the development of nuclear fragmentation and of epithelial loosening. It has not

been possible, so far, to specify precisely in what respects the oxidative processes are abnormal. The available evidence is discussed in detail in the papers by Dr. Herrmann, and in the final summary (page 326).

A prominent feature of corneal metabolism revealed in this work is the metabolic interaction between epithelium and stroma. The epithelium was found to have a carbohydrate metabolizing system similar to that of many other tissues involving a cyanid sensitive oxygen acceptor, hexose phosphorylation and cleavage. The stroma has no measurable oxygen uptake but can consume glucose at a rate per cell twice that of the epithelium. Part of the glucose utilized can be recovered as lactate but the tissue has no power of utilizing lactate. In the isolated cornea the lactate produced by the stroma is consumed by the epithelium, in spite of the fact that the concentration of lactate is generally higher in the epithelium than in the stroma. Conclusive proof that lactate is actively transferred from stroma to epithelium is lacking, but the utilization of the stroma lactate by the epithelium is completely inhibited by mustard poisoning. Similar results were obtained with serine which is also utilized by the epithelium but not by the stroma.

It is to be emphasized that the interference by mustard in the intercellular metabolism is not all embracing. Pyruvate injected into the stroma is utilized at a normal rate after mustard poisoning. Glucose utilization is also not inhibited. Nor is interference with the intercellular metabolism the sole result of mustard injury. Quite obviously, the tissues even if mechanically separated from one another are susceptible to injury from mustard. We wish merely to point out that one of the aspects of mustard injury is a disturbance in the intercellular metabolism of the cornea and that mustard furnishes a powerful tool for the study of this hitherto poorly explored field of tissue metabolism.

In one large field our attempt to study corneal metabolism met with complete failure. It was hoped that by using nitrogenous metabolites containing N^{15} we would be able to study the course of synthetic activities in the tissue. A mass spectrograph was placed at our disposal by the Department of Chemistry of the Johns Hopkins University for this purpose and considerable funds were used in an effort to adapt this instrument to our purposes. Unfortunately,

the instrument was designed for high resolution and yielded an ionic stream of extremely low amperage. In spite of much effort it was found impossible to adapt this instrument for quantitative estimation of N^{15}/N^{14} ratio in samples, and the effort had to be abandoned. The difficulties here were wholly technical and there is no reason why work in this field will not some day yield important results. Indeed, preliminary runs analyzed for us by Dr. Rittenburg of Columbia University showed that the nitrogen turnover of a single isolated beef cornea yielded measurable amounts of protein synthesis.

PERSONNEL

Mention has been made above of the special contributions of several of the individuals in our group, but this by no means suffices to acknowledge the share of various others nor adequately to record the uniform enthusiasm and devotion of the whole group. The appended table 1 lists all of the members and the main field of activity of each.

COOPERATION WITH OTHER GROUPS

- 1) Department of Physiological Chemistry, Johns Hopkins Medical School. In undertaking work in the field of chemical warfare with which we had no previous acquaintance, need was felt for help from more experienced colleagues. Dr. W. M. Clark, Professor of Physiological Chemistry at the Johns Hopkins Medical School, generously placed the cooperation of his staff and the facilities of his department at our disposal. In order to protect the patients in the Wilmer Institute from risk, stock supplies of toxic agents were stored in the laboratory of Physiological Chemistry under the care of Dr. Curt C. Porter. Dr. Leslie Hellerman and Dr. Barnett Cohen gave most generously of their time and thought to our problems. Dr. Hellerman accepted the position of consultant for our group and on several occasions performed a number of titrations and preparations for us. In this he was assisted at one period by Mr. Presta, and later by Dr. M. R. Bovarnick.
- 2) Other Ophthalmological Groups. During the course of the work, conferences were held from time to time with the other ophthalmic groups working in this field. These conferences were arranged by the Committee for the Treatment of Gas Casualties and proved

extremely useful in dividing up the domain of study, in arranging mutual checks in respect to special experiments, in occasional direct collaboration, and particularly in formulating the conclusions of the various studies in terms of draft directives for use by the armed forces.

TABLE 1

NAME	FULL OR PART TIME	LENGTH OF SERVICE	TITLE	CHIEF FIELD OF ACTIVITY
1. Dr. Alan C. Woods		11/1/41-12/31/45	Responsible investigator	In charge of administration.
2. Dr. Jonas S. Friedenwald		11/1/41-12/31/45	Responsible investigator	In charge of research.
3. Dr. Roy O. Scholz	Full	4/1/42-7/1/45	Ophthalmologist	Pathology and treatment of mustard burns.
4. Dr. Wm. F. Hughes, Jr.	Full	4/1/42-7/1/43	Ophthalmologist	Pathology and treatment of Lewisite burns.
5. Dr. Albert Snell, Jr.	1/2	7/1/44-12/31/45	Ophthalmologist	Screening test for mustard antidotes.
6. Dr. Alfred E. Maumenee	Full	9/1/43-10/1/44	Ophthalmologist	Pathology and treatment of nitrogen mustard.
7. Dr. Jack S. Guyton	Part	1/1/43-1/1/44	Ophthalmologist	Secondary infections.
8. Dr. Wilhelm Buschke	Part	1/1/43-12/31/45	Ophthalmologist	Effect of mustard on the mitotic activity and nuclear fragmentation in the corneal epithelium.
9. Dr. Heinz Herrmann	Full	7/1/42-12/31/45	Chemist	Effect of mustard on corneal metabolism; loosening of the corneal epithelium.
10. Dr. Samuel A. Talbot	Half	7/1/43-7/1/45	Physicist	Mass spectrograph for N ¹⁴ estimations.
11. Dr. Leslie Hellerman	Part	7/1/42-12/31/45	Chemical consultant	Consultant.
12. Mr. Ulric A. Presta	Part	7/1/43-10/1/43	Chemist	Assistant to Dr. Hellerman.
13. Dr. Marianna R. Boverinch	Part	10/1/43-1/1/44	Chemist	Assistant to Dr. Hellerman.
14. Dr. Earl L. Burky	Part	4/1/42-4/1/43	Bacteriologist	Secondary infections.
15. Fay H. Hickman	Full	4/1/42-10/1/44	Technician	Assistant to Dr. Herrmann.
16. Jane E. Crowell	Full	6/15/43-7/1/45	Technician	Assistant to Dr. Friedenwald and Dr. Buschke.
17. Dorothy Breeskin	Full	7/1/45-12/31/45	Technician	Assistant to Dr. Friedenwald and Dr. Buschke.
18. Sylvia G. Moses	Full	8/1/43-7/1/45	Technician	Assistant to Dr. Herrmann.
19. Grace Fitcher	Half	7/1/43-12/1/43	Technician	Assistant to Dr. Talbot.
20. Nathalie Weisgall	Half	11/1/43-7/1/45	Technician	Assistant to Dr. Talbot.

The exchange of ideas between the various groups proved mutually stimulating.

3) Committee on Physiological Mechanisms of NDRC. The close relation between our work and that of the Committee on Physiological Mechanism of the NDRC was recognized early and arrange-

ments for mutual exchange worked out. The Committee invited representatives of our group to attend the regular scientific meetings of its contractors and to report, on occasion, phases of our work that were relevant to their interests. These meetings and the informal exchanges that surrounded them were extremely useful both in respect to the exchange of ideas and in arrangements for complementary and collaborative undertakings.

4) Research Laboratory of duPont. Shortly after the development of our screening test for prospective mustard antidotes, it became apparent that considerable synthetic work in the preparation of compounds for testing would be desirable. Arrangements were made by Dr. Winternitz and Dr. Marvel for a contract with the Chemical Department, Experimental Station of the E. I. duPont de Nemours Co. through the 9-1 Division on Organic Chemistry of NDRC. The work at duPont was conducted first by Dr. Lazier and later by Dr. Howk. Almost 100 compounds were prepared. Both Dr. Lazier and Dr. Howk and their associates gave most thoughtful consideration to the problem and made numerous suggestions which became incorporated in the work. Dr. Marvel and Dr. Adkins, his successor as chairman of the 9-1 Division, were continuously helpful.

5) Edgewood. Cooperation was arranged with the staff at Edgewood Arsenal on a number of field tests and surveys of exposed personnel. In addition, Major Laughlin, Chief of the Division of Ophthalmology of the Medical Research Laboratory at Edgewood, was throughout the period of joint work continuously in close touch with our laboratory, and on a number of occasions performed checking experiments in relation to some of our studies.

SUMMARY AND DISCUSSION

The coordinated study which has been outlined above fell far short of its goal. No efficacious method for the treatment or prevention of mustard injury of the eye was achieved as the result of our work. On the basic problems of the physiology, biochemistry, toxicology, and cytopathology of the cornea we feel that we have done little more than scratch the surface. For a deeper penetration into these fields much more time and many more techniques will no doubt be required. Our reasons for publishing the relatively unconnected aspects of this study

in a single issue and for presenting the outline of the work in this introductory chapter, are that we hoped in this way to point out the interrelations, however tenuous, between the different parts of the investigation. The mutual fertilization involved in this coordinated effort was the factor chiefly responsible in enabling us to open up several new fields of experimental study.

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II. PRIMARY REACTION OF MUSTARD WITH THE CORNEAL EPITHELIUM*

JONAS S. FRIEDENWALD, ROY O. SCHOLZ, ALBERT SNELL, JR., AND SYLVIA G. MOSES

Studies have been undertaken by a number of investigators to determine tissue components that react with mustard. There is general agreement that the tissue proteins are chiefly concerned in these reactions and many enzymes have been shown to be inactivated by reaction with mustard. Various reactive groups normally present in proteins, particularly sulfhydryl, amino, and carboxyl groups have been shown capable of reacting with mustard, and in homogenous solutions sulfhydryl compounds show the highest competition factors among the biochemical substances. Presumably, therefore, a part of the mustard bound by tissue is bound by sulfhydryl side chains of proteins. Studies with mustard synthesized from radioactive sulfur have in the hands of Henriquez (1) revealed much useful information as to the proportion of reacting mustard which is bound by the skin, the rate at which penetration and binding takes place, and location of bound mustard in the affected tissue. Similar studies by Hamilton and Axelrod (2) have added much to our knowledge of similar factors in respect to the cornea. The resolving power of radio histograms, however, is inadequate to reveal individual cells. The following investigations were undertaken with the aim of throwing light on the primary reaction of the mustard with the tissue. It cannot be claimed that these studies have given an answer to this question. The results, however, are of some interest and some of the methods used may have potential applicability to other cytochemical fields.

I. AMOUNT OF MUSTARD IN CORNEA

In the experiments to be reported in this and in subsequent papers eyes were exposed to mustard vapor at room temperature (22-24°C.) generally in one of two ways. In some experiments the eyes were placed over the mouth of a cylindrical weighing bottle 5 cms deep,

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

the bottom of the bottle being covered by a layer of liquid mustard. Dosage was varied by varying the time of exposure. This technique was, in general, used for exposure of enucleated beef eyes. In other experiments the eyes were placed over the mouth of a similar cylindrical vessel to which a small bore glass tube was attached as a side arm (see Fig. 1). A slender wooden stick (swab stick) was inserted through the tube and a small piece of filter paper inserted in the end of the stick within the vessel. Mustard was placed not only on the floor of the vessel but also on the filter paper. By rotating the stick a fanning action of the filter paper could be produced and the air in the chamber kept saturated with mustard vapor. The vessel was pro-

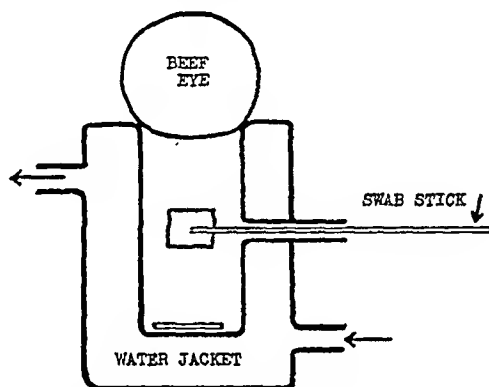


FIG. 1. CHAMBER FOR EXPOSURE OF CORNEAS TO SATURATED MUSTARD VAPOR

vided with a double wall through which water could be circulated to keep the temperature constant. Dosage could be controlled either by varying the time or the temperature of exposure.

It was desirable to find out how much mustard entered the cornea per minute with these types of exposure. Experiments were performed in which circular buttons of beef cornea, of known area, cut with a cork borer were placed on the under surface of the glass stopper or shallow vessels (2.0 cm. deep) of the first type (i.e. without the fan). After exposures of varying periods the buttons were removed and immediately immersed in isopropyl alcohol for 20 minutes, during which time they were ground and pressed intermittently. Further extraction did not increase the recovery significantly. At the conclusion of the period of extraction, a 2.5 ml. portion of the isopropyl

alcohol extract was used for the colorimetric determination of its mustard content by a procedure not yet released for publication. The lower limit of sensitivity of the method is about 2 micrograms in 5 ml. The results of these titrations are charted in Fig. 2. Extrapolating the curve to zero time and drawing the tangent to the curve at the origin one obtains an estimate of 3.3 micrograms uptake of mustard per square centimeter per minute.

In a second series of experiments the corneal buttons after exposure to mustard vapor for 20 minutes were transferred to another similar but empty vessel and allowed to remain in this vessel at room temperature for varying periods before being immersed in isopropyl alcohol. These titrations afforded an estimate of the rate at which mustard

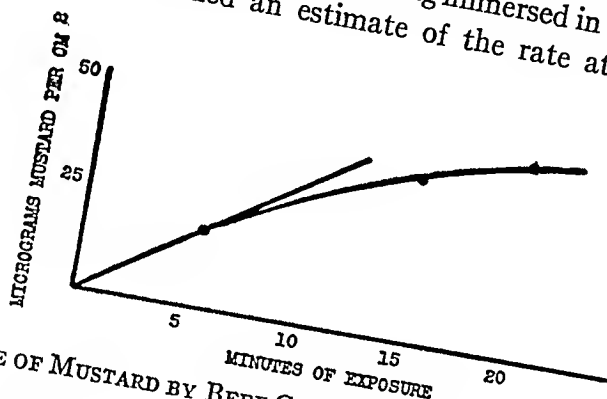


FIG. 2. UPTAKE OF MUSTARD BY BEEF CORNEA EXPOSED IN 2 CM. DEEP CHAMBER

reacts with the tissue. The results are shown in Fig. 3. Extrapolating this curve back to zero time and drawing the tangent to the curve at its beginning one reaches the conclusion that about 8% of the mustard in the cornea disappears per minute. Referring back to Fig. 2, it is seen that the concentration of mustard in the cornea on continuous exposure to vapor under these experimental conditions tends toward a maximal level of about 50 micrograms per square centimeter. At this steady state the rate of uptake should equal the rate of disappearance. The rate of disappearance is 8% per minute or 4 micrograms per cm² per minute. These two methods therefore give reasonably good agreement.

Similar experiments with deeper vessels (5 cm. deep) yielded an uptake of 0.6 micrograms per cm² per minute, while experiments with vessels with the fan (saturated mustard vapor) gave an uptake of 4.0

micrograms per cm^2 per minute, and exposure to liquid mustard showed an uptake of about 8 micrograms per cm^2 per minute. All of these experiments were performed at 22°C .

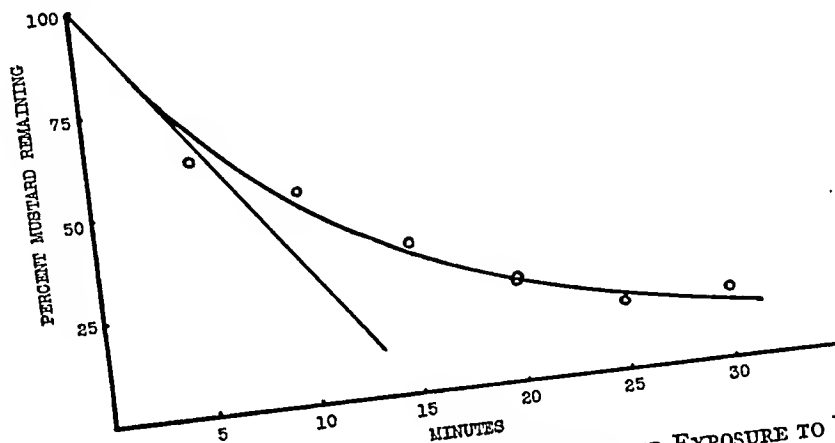


FIG. 3. DISAPPEARANCE OF MUSTARD FROM CORNEA AFTER EXPOSURE TO VAPOR

TABLE I
Relation of Mustard Uptake to Effects Observed

MICROGRAMS. MUSTARD PER CM^2	RABBIT	RAT	BEEF
10	Cornea perforates Permanent scarring Loosening of epithelium	Cornea perforates	Threshold for loosening of epithelium and metabolic effects
1		Permanent scarring Loosening of epithelium	
0.1		Threshold for nuclear fragmentation	
0.01		Threshold for mitosis inhibition	

Using these figures together with experimental results to be reported below, it is possible to estimate the amount of mustard required to produce various corneal symptoms. The results are shown in Table I.

These data may be elaborated further with the aid of the following considerations. The studies (2) have shown that only about 15% of the mustard which reacts in the tissue becomes bound, presumably

by reacting with fixed elements of the tissue. The remainder is extractable, presumably in the form of thiodiglycol, and hence presumably having reacted with tissue water. Thus the effective portion of the absorbed mustard may be estimated as 15% of that indicated in the table. Since the major portion of the bound mustard was shown to be fixed in the epithelium, the following calculations can be made. At the threshold of mitosis inhibition for the rat's cornea, .01 micrograms of mustard per cm^2 are required. Of this .0015 micrograms epithelial cells per square centimeter. Thus the effective dose to produce minimal mitosis inhibition is of the order of 2.5×10^{-10} micrograms per cell, or 1.5×10^{-18} gram moles per cell, or, using Avogadro's number 10^6 molecules of mustard per cell.

II. GLUTATHIONE AND MUSTARD IN THE CORNEAL EPITHELIUM

Beef corneal epithelium has been shown (3) to contain quite large amounts of glutathione, roughly 150 micrograms per cornea or 40 micrograms per square centimeter. Like other sulfhydryl substances glutathione has a high competition factor for mustard and yields a nontoxic compound as the resultant of this reaction. It was of interest, therefore, to discover how much of the mustard absorbed by the cornea reacted with glutathione.

Procedure and Results: Glutathione was extracted from whole beef corneas or from corneal epithelial cell preparations with sulfosalicylic acid and titrated colorimetrically with the aid of the nitroprusside reaction. Previous studies reported elsewhere (3) have shown that this method is adequately specific when applied to this tissue, and that practically all of the measurable glutathione in this tissue is contained in the epithelium. Sulfosalicylic acid was added in such amounts as to constitute 2.3% of the extracting solution. Mustard alone gave no color reaction in the nitroprusside test.

Studies reported in the previous section showed that under the conditions of exposure to mustard which we used, approximately 70 micrograms of mustard were taken up per beef cornea in half an hour.

1) Intact beef corneas were exposed to mustard vapor for $\frac{1}{2}$ hour in the deep cylindrical vessels previously described. Pairs of eyes carefully matched as to corneal size were used for treatment and

controls. After exposure, the treated and control corneas were incubated for one hour and then extracted with sulfosalicylic acid. No significant difference in glutathione content was noted between the treated and control samples, though both showed less glutathione than fresh unincubated specimens.

2) Beef eyes of uniform size were selected for each experiment, and the epithelial scrapings of 4-5 corneas pooled for each sample. This amount of tissue contained about 0.6 mg. glutathione. The scrapings were ground in a mortar and suspended in 4.9 ml. Ringer's phosphate solution, and 0.1 ml. of a solution of mustard in alcohol containing 2

TABLE II

Recovery of Glutathione from Suspensions of Corneal Epithelium in Ringer's Phosphate Exposed to 2 Mg. Mustard for 30 Minutes

CONTROLS GLUTATHIONE ALONE	TISSUE SUSPENSION	TISSUE SUSPENSION + 2 MG. MUSTARD	TISSUE SUSPENSION + GLUTATHIONE	TISSUE SUSPENSION + GLUTATHIONE + 2 MG. MUSTARD
Milligrams Glutathione Recovered				
Complete recovery	0.48	0.40	3.7	2.0
	0.48	0.43	3.0	1.75
	difference not significant		3.0	1.6
			3.0	1.85
			3.0	1.7
			Av. difference = 1.36 mg. or 45.3%	

mg. mustard was added. In some cases 2 mg. glutathione was also added. Samples and controls without added mustard were allowed to stand for 30 minutes at room temperature, when precipitated with sulfosalicylic acid. A slight, but not significant, disappearance of glutathione was noted in the epithelial cell emulsions treated with mustard, though when glutathione had been added there was marked diminution on exposure to mustard.

3) Similar samples of corneal epithelium were next ground in a tissue grinder with broken glass, suspended in Ringer's phosphate solution, and then treated with 2 mg. mustard. The results in these experiments showed a significant drop in titratable glutathione. Some of these samples were frozen immediately after scraping, thawed

in about an hour, refrozen and thawed again, and then ground with powdered glass in a tissue grinder. Smears from these preparations showed complete disintegration of all cellular and nuclear structure. After exposure to mustard a significant drop in glutathione content was found.

TABLE III
Recovery of Glutathione from Preparations of Completely Macerated Corneal Epithelium Exposed to Mustard

TISSUE SUSPENSION ALONE	TISSUE SUSPENSION + 2 MG. MUSTARD	DIFFERENCES
.35 mg.	.20 mg.	.15 mg.
.48 mg.	.30 mg.	.18 mg.
.65 mg.	.40 mg.	.25 mg.
.55 mg.	.41 mg.	.14 mg.
.55 mg.	.20 mg.	.35 mg.
	.20 mg.	
Average difference = .21 mg. or 41%		

TABLE IV
Recovery of Glutathione after Treatment with Mustard for 30 Minutes

2 MG. GLUTATHIONE IN RINGER'S PHOSPHATE	2 MG. GLUTATHIONE IN RINGER'S PHOSPHATE + 2 MG. OF AN ALCOHOLIC SOLUTION OF MUSTARD	DIFFERENCE
mg.	mg.	mg.
2	1.10	0.9
2	1.60	0.4
2	1.10	0.9
2	0.95	1.05
2	1.10	1.0
	0.9	1.1
Av. difference = .89 mg. or 44.5%		

4) Control experiments were performed with glutathione in Ringer's phosphate solution with and without added mustard. After standing at room temperature for $\frac{1}{2}$ hour sulfosalicylic acid was added and titrations for glutathione performed. The proportion of glutathione which disappeared in these solutions was of the same order as in those of paragraph three above. It is concluded that in the intact cell glutathione is relatively in-

accessible to reaction with mustard. This might be due to the presence of strongly competing factors in the tissue, but this is unlikely because mere grinding of the cells removes this protection. A second possible explanation is that the glutathione is protected by having its sulfhydryl group blocked through some reversible chemical association in the tissue. This is also unlikely since grinding of the tissue would not be expected to reverse such an association. A third possibility, and one which appears most likely, is that the glutathione normally resides in a region within the cells to which mustard has poor access. This conclusion is the more remarkable when considered in relation to the fact to be shown in subsequent papers of this series, that with the doses of mustard used in these experiments many cellular processes are inhibited 100%. One must conclude that cellular components concerned in these inhibited processes either compete for mustard much more intensely than does glutathione or that they lie in regions much more accessible to mustard. The latter would appear the more likely.

III. ELECTRICAL RESISTANCE OF THE CORNEA

The experiments reported in the previous section strongly suggest that mustard does not distribute itself homogeneously throughout the affected cells but may, on the contrary, present itself in much higher concentrations in some intracellular regions than in others. Since mustard is much more lipoid soluble than water soluble it may be supposed that the initial distribution of the agent would occur through solution in the lipoid components of the cells. Moreover, mustard does not become reactive until activated by water. Consequently, those cellular components adjacent to lipoidal structures might be expected to receive the heaviest dose of the agent. These considerations are supported by the fact that the rate of disappearance of free mustard in tissue is considerably slower than in homogeneous aqueous solutions and is similarly retarded in aqueous emulsions of lipoids such as lecithin.

In view of these possibilities it appeared desirable to determine whether the primary reaction of mustard with the cornea was associated with any demonstrable change in the cell membrane. The electrical resistance of the cornea depends on the impermeability of the

epithelial boundaries to electrolytes and consequently may be used as an index of the integrity of the cell membrane. It is to be admitted that the argument just outlined loses some of its cogency when one considers the fact that the nitrogen mustards which even at physiological pH are much more water soluble than mustard nevertheless produce an almost identical pathological effect. The following experiments were performed in cooperation with Dr. Curt Richter and Mrs. Betty Woodruff.

Methods: Current was applied through silver-silver chloride electrodes connected by a tube of physiologic saline to agar plugs made up in physiologic saline. The ends of the agar plugs which were brought in contact with the tissue were trimmed down to a determined area and shape. The resistance of the electrode circuit and ammeter was less than 200 ohms. Increasing voltage was applied until the current reached 2 milliamperes and the voltage necessary to produce this current was measured by a volt meter and used to calculate the resistance (4).

In preliminary experiments an electrode with a circular surface 2 mm. in diameter was placed in contact with the cornea of each eye of a rat either under ether anaesthesia or freshly killed by decapitation. The resistance measured was about 100,000 ohms. When the epithelium was scraped off one cornea and the electrodes reapplied the resistance dropped to about 50,000 ohms, and when the epithelium had been scraped off both the corneas the resistance was less than 500 ohms. Similarly, when a freshly enucleated eye was placed with one electrode in contact with the corneal epithelium, and one in contact with the sclera, resistance of 50,000 to 200,000 ohms was measured, but if the epithelium was scraped off the resistance was less than 500 ohms. Similar results were obtained when the isolated cornea was placed with its endothelial surface in contact with one electrode, its epithelial surface in contact with the other. It seems clear that only the corneal epithelium contributes an appreciable resistance to the flow of current. No difference could be measured depending on the direction in which the current was applied.

Very large variations in resistance were found to occur with variations in the state of hydration of the tissues. If freshly enucleated rats' eyes were placed in 0.9% sodium chloride solution for 15 minutes

or more the electrical resistance dropped markedly. If the eyes were allowed to dry at room temperature the resistance rose markedly. This was not due simply to the presence or absence of a surface film since a partially dried cornea maintained its high resistance even if the surface was washed with saline immediately before the measurement. In the experiments reported below this variation was largely eliminated by placing the treated and control eyes together in closed moist chambers with the eyes resting on filter paper soaked in physiologic saline solution. Under these circumstances the resistance in the control eyes was stable for an hour or two and then slowly fell. When two eyes of the same animal were compared the resistance was found to be similar (within 30%). In different animals the resistance varied from 100,000 to 500,000 ohms per square millimeter.

Rats' eyes were exposed to mustard vapor either *in vivo*, or *supravivally* by suspending freshly enucleated eyes in a chamber saturated with mustard vapor. Exposures were varied up to levels sufficient to produce lesions of maximal severity. For comparison other eyes were exposed to iodoacetate, fluoride, cyanide, and ultraviolet light. Resistance was measured before and immediately after exposure to mustard and to these various other agents, and in no case was any consistent change in resistance noted different from that found in control eyes exposed to solutions or chambers free from toxic agents. On incubation in moist chambers the resistance of both control and treated eyes slowly declined for several hours. When the epithelium began to become loosened after several hours of incubation following exposure to mustard, fluoride, iodoacetate, and ultraviolet light (but not after cyanide) the resistance dropped sharply, and when the epithelium sloughed off the resistance dropped to minimal levels.

It is concluded that exposure to mustard does not result in any primary injury to the corneal epithelial cell membrane detectable by measurements of the electrical resistance of the tissue. The conclusion that the cell membrane is not a primary site of mustard injury was supported by the results of experiments on red blood cells performed in collaboration with Dr. Wintrobe. Neither the fragility nor the sedimentation rate of red blood cells was altered on exposure to mustard in doses as high as 0.1 mg. per ml. Furthermore, Adrian (5) has reported that action currents in nerve fibers exposed to mustard remain normal.

IV. HISTOLOGICAL STUDIES

The following study was undertaken in a further effort to discover the intracellular sites of the primary reaction of mustard. Since mustard has been shown to combine with various reactive groups of proteins it seemed possible that the acid-base characteristics of the reacted proteins might be altered and that this alteration might exhibit itself in some change in the acid-base staining characteristic of the tissue. In order to explore this hypothesis it was necessary to develop quantitatively controlled methods of staining and of measuring the amounts of stain bound by the tissue.

Methods: The eyes of young adult rabbits were used in these experiments. One eye was exposed to saturated mustard vapor, the other eye was used as control. Severe contaminations were produced by exposures of from 30 minutes to 1 hour to saturated vapor at 24°. The eyes were removed thirty minutes later. At this time no marked histo-pathological changes were to be found on routine histological study.

In the first experiments fixation was achieved by a modification of the Altmann-Gersh (6) freezing-drying technique. Immediately on enucleation the tissue was frozen by immersion in absolute alcohol which had been cooled by solid carbon dioxide. The container and tissue were then transferred to the freezing compartment of an electric refrigerator and maintained at a temperature of -11° for 3 days. This procedure produced good fixation and dehydration. However, specimens fixed in Zenker's solution gave the same histological results. Consequently Zenker fixation was used in most of the later experiments.

The tissue blocks were embedded in paraffin, and sections 7 microns thick were cut and mounted on glass slides. The paraffin was removed with xylol, and the sections were then passed through a series of alcohol water mixtures and washed in water. The slides were then immersed in dilute solutions of dyes dissolved in M/16 buffer solutions. Acetate buffers were used for the range of pH 2.0 to pH 5.0, phosphate buffers from pH 5.0 to pH 7.0, and borate buffers from pH 7.0 to pH 13.0. Very dilute solutions of dye, of the order of .004 to .008% by weight, were used. The tissue and dye were equilibrated over a 24 hour period at room temperature, and were then mounted in the dye

solution covered by a cover slip. Estimate of the intensity of the staining was made with the aid of a simply constructed colorimeter attached to a microscope.

This apparatus was constructed with standard microscope lamps, microscope, camera lucida, a rack and pinion, lucite wedge containing solutions of dye, and a slit and focusing lens. The optical system is shown in Fig. 4. A square diaphragm was inserted in the ocular focal

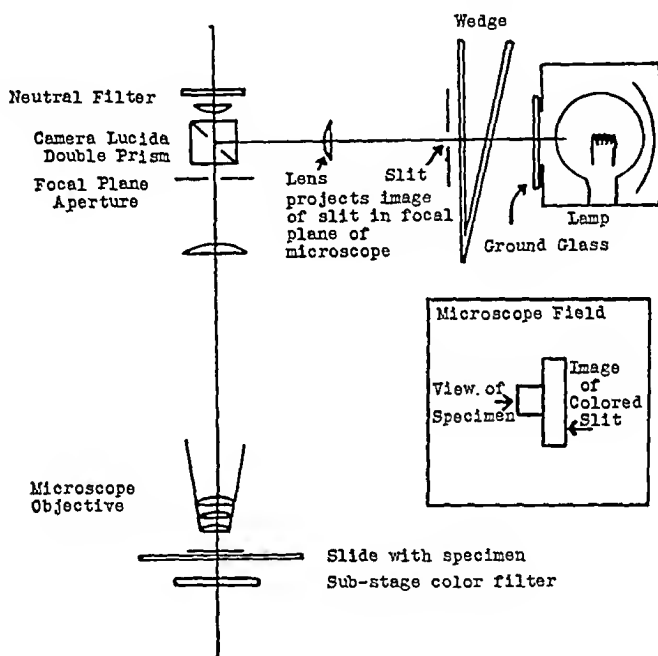


FIG. 4. OPTICAL SYSTEM OF MICROSCOPE-COLORIMETER
Inset shows field as seen through microscope ocular.

plane of the microscope so that only a few cells could be viewed at a time. The camera lucida prism was arranged so that the image of the illuminated rectangular slit could be focused immediately adjacent to the square aperture filled with the images of the cells being studied. The intensity of color in this rectangle could be varied by moving the wedge up and down behind the slit. An arbitrary scale was placed on the rack and pinion so that a numerical value could be given each reading.

Before each set of readings a region of the microscopic slide prepara-

tions which contained no tissue was focused in the field of the microscope and the lucite wedge was raised so that the colorless portion of the wedge below the dye chamber was behind the slit. The microscope lamp, substage aperture, and, if necessary, color filters were arranged so that a good match in brightness and hue was obtained in the two comparison fields. Some dyes in solution do not give good color matches with the same dyes in histological stains. When this occurred the color in the wedge was adjusted to match that in the sections by using appropriate dye mixtures. For instance, crystal violet in stained sections has a more bluish color than in solutions of the same pH. An appropriate bluish color can be achieved within a small range of variation by lowering the pH or the concentration of the crystal violet solution in the wedge. The same result can be achieved more easily by using mixtures of crystal violet and methylene blue in the wedge. Since we were concerned only with relative color intensities in various sections and various portions of the same sections, no special attention was given to the absolute concentration of the dyes in the comparison wedge, but only a convenient concentration was chosen which would give readings in the middle range of the wedge. The same standard solution, was, of course, used throughout for any given experimental set of readings.

Three readings were taken on each section and two or three sections stained with each dye buffer mixture were read. After all of the slides had been measured the labels were uncovered and the readings for similarly prepared slides were averaged. The sections were stained with either basic or acid dyes, but not with both together. The stains were not "differentiated," i.e., washed in decolorizing solutions, but were mounted without washing in the same dye solution in which they had been stained.

Results: Crystal violet was the basic dye of choice. When normal controls were stained it was found that the intensity of the stain increased in the epithelium and stroma as the pH of the dye solutions was increased (Fig. 5). As the pH increased from 3.5 to 6 metachromasia of the tissue became more marked. When the stainability of control corneas was compared with contaminated corneas no difference could be found at low pH. At pH above 6.0 the staining intensity was so great that accurate readings became difficult. It was, therefore, impossible to extend the study of the basophilic staining of the

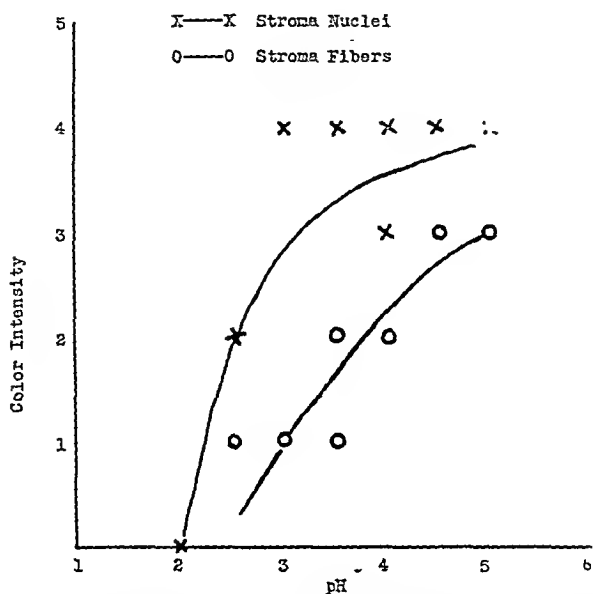


FIG. 5. SAMPLE OF MEASUREMENTS OF STAINING INTENSITY WITH .0047% CRYSTAL VIOLET SOLUTION

BUFFER SYSTEM

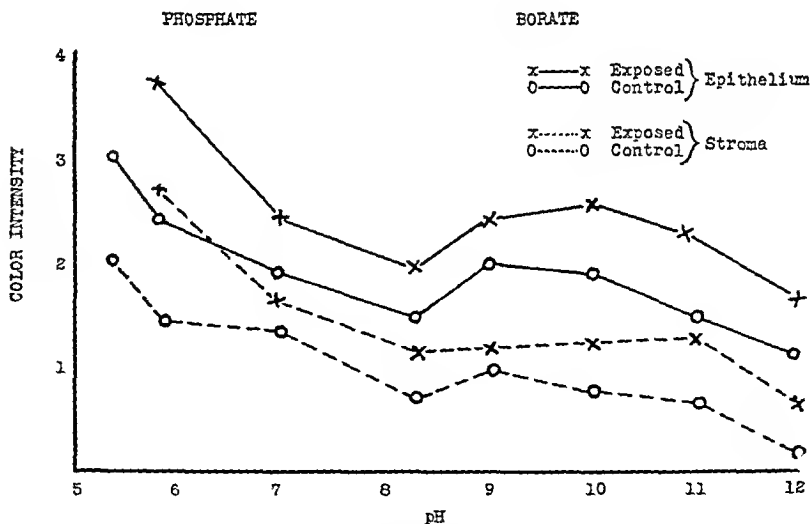


FIG. 6. STAINING INTENSITY WITH .008% PHLOXINE AFTER ONE HOUR EXPOSURE TO SATURATED MUSTARD VAPOR

tissue to the pH regions at which the sulfhydryl groups of proteins might be expected to dissociate.

In order to study the acidophilia of the tissue the eosin family was investigated. Phloxine was selected because the color produced was the most easily readable. Figure 6 shows the intensity of dye in the sections stained at various pH. At pH 5 and below color matching was difficult and the dye in the buffer solutions is partially or completely precipitated. In all sets of experiments with phloxine a broad hump in the curve is observed around pH 10. It would seem likely that in this region the phenolic group of phloxine becomes dissociated and that the resulting dibasic acid has a higher affinity for the tissue than the monobasic acid present at lower pH, though no color change in phloxine solutions is noted in passing through this pH range. Com-

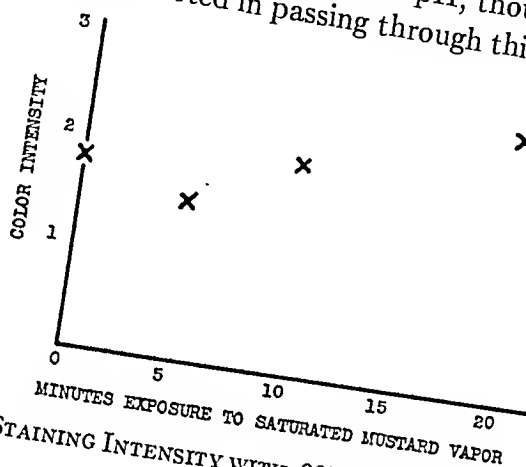


FIG. 7. STAINING INTENSITY WITH .007% EOSIN B AT pH 7.5

paring the graphs of the contaminated tissues with those of the controls, it is seen that throughout the pH range studied the contaminated tissue takes up more dye than does the control. The increased stainability is apparent when less severe exposures to mustard are used, but is no longer detectable after only 5 minutes exposure or less (Fig. 7).

In one set of experiments the control and contaminated sections were immersed in a 5% solution of potassium cyanate at pH 7 for 24 hours, rinsed, and then stained with phloxine at pH 6.0 and pH 7.0. The controls showed a smaller dye uptake than controls not treated with cyanate. The mustard contaminated tissues also showed less acidophilia after cyanate treatment than without this treatment, but they still showed the same excess over the controls as in experiments in which cyanate was not used (Table V). The increased acidophilia of tissues after mustard contamination

indicates that in the reaction of mustard with tissue either new basic groups are formed or acid groups are suppressed. If carboxyl groups in appreciable numbers were suppressed, some decrease in the basophilia of the tissue should have been noted at pH below 6 but this was not the case. Reaction of mustard with sulfhydryl groups would, of course, diminish the number of these groups capable of dissociating to form anionic side chains at pH 8 and above. However, the increased acidophilia is already apparent well below the dissociation point of the common sulfhydryls of the tissues. Furthermore the increased acidophilia is also seen in Zenker fixed tissues. It is most doubtful that any sulfhydryl groups survive in the reduced state after Zenker fixation and the subsequent washing in Lugol's solution which

TABLE V

Intensity of Phloxine Stain in Epithelium Treated with KCNO and Controls

Corneas exposed to saturated mustard vapor for 20 minutes at 22°C. Sections immersed in 5% KCNO at pH 7.0 for 24 hours before staining.

	pH 6.0	pH 7.0
1. Exposed, treated with KCNO.....	1.4	1.5
2. Control, treated with KCNO.....	1.1	1.1
3. Exposed, no KCNO.....	1.6	
4. Control, no KCNO.....	1.3	

is necessary to remove the precipitated mercury. Consequently, the increased acidophilia of the tissue cannot be attributed to the suppression of SH acid dissociation.

Furthermore, the persistence of the increased acidophilia after cyanate treatment indicates that the basic groups involved are not primary nor secondary amines, and its persistence at high pH indicates that the basic groups involved are relatively strong bases. These considerations lead to the suggestion that the change in the staining reactions of the tissue may be due to the formation of alkyl-sulphonium groups in the tissue in its reaction with mustard.

With the staining technique used control tissues take up considerable amounts of dye, and a difference between mustard treated tissues and controls could be observed only after massive exposures to mustard. With these massive doses no special intracellular locations of enhanced

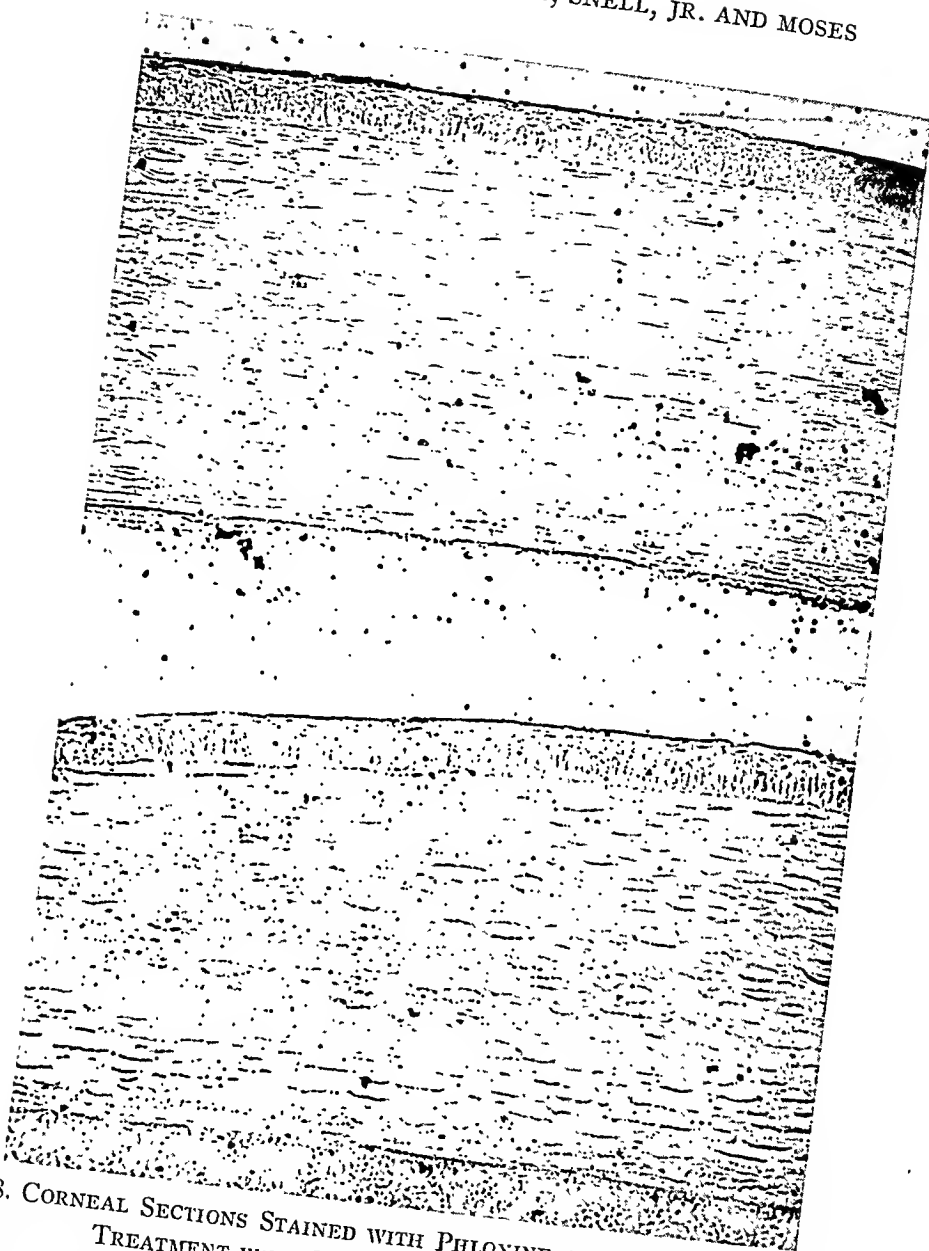


FIG. 8. CORNEAL SECTIONS STAINED WITH PHLOXINE AT PH 10 AFTER PREVIOUS
TREATMENT WITH GLYOXAL AND WITH NITROUS ACID
Above: Control.
Below: Exposed to 20 mg. mustard per liter for 20 minutes.

eosinophilia could be recognized. The experiments reported above with potassium cyanate suggested that the non-specific staining of the tissues might be reduced by special treatments leading to an en-

hanced contrast between the mustard treated tissues and the controls. It was found that if the sections were exposed to glyoxal and then to nitrous acid the phloxine binding capacity of the controls was greatly reduced without affecting the excess stain uptake of the mustard exposed tissues. The experiments were performed as follows:—

After removal of the paraffin, sections were placed in a 5% solution of glyoxal in phosphate buffer pH 7 for 24 hours, washed in water and then placed in a solution containing 10 grams KNO_2 plus 10 ml. glacial acetic acid in 80 ml. water, also for 24 hours. After thorough washing they were stained in a .004% solution of phloxine at pH 10, and mounted in this dye solution. Control tissues were almost free of the red color of phloxine but were slightly yellowish presumably as the result of the formation of nitro compounds. When viewed through a yellow color filter they appeared almost colorless. Tissues that had been exposed to saturated mustard vapor for 30 seconds or longer showed an appreciably enhanced acidophilia, but with these doses the stain was still relatively diffuse and no special intracellular sites for the enhanced eosinophilia could be distinguished. The effort was therefore made to push the experiments to the extreme limit of sensitivity of the method.

With the kind cooperation of Dr. Silver at Edgewood Arsenal rabbit eyes were exposed to a concentration of 20 milligrams of mustard per liter for 20 minutes. This is approximately equivalent to an exposure to saturated mustard vapor at 24° for 3 seconds. Exposed and control eyes were fixed in cold alcohol or in Zenkers and sections treated with glyoxal and nitrous acid before staining with phloxine. The difference between the treated and the control specimens was almost imperceptible but the region of the nuclear membrane and the basement membrane appeared slightly more intensely stained in the treated than in the controls, suggesting that these may be among the sites of primary mustard reaction.

Discussion: Estimates have been presented of the amount of mustard taken up by the cornea after various types of exposures. These studies were undertaken as a guide in the interpretation of the experiments that were to follow. In the succeeding papers in this series it will be shown that, as the dosage of mustard is increased, one tissue process after another becomes inhibited, one symptom after another

becomes manifest. More than a thousand fold variation in dosage is required to cover the range from the most sensitive to the most resistant effects. Yet even with massive doses capable of producing complete necrosis of the tissue, some tissue components such as glutathione, which in vitro are among the strongest biochemical competitors for reaction with mustard, still fail to react in measurable quantity. These facts and the experiments reported above point strongly to the conclusion that mustard may distribute itself within the cells in a highly unequal manner, finding ready access to some regions while other regions are relatively inaccessible to it. Our search for the intracellular sites of most intense mustard reaction has been largely inconclusive. There is no evidence that the cell membrane is particularly susceptible to mustard damage, but there is some evidence, regrettably feeble and inconclusive, that the region of the nuclear and basement membranes may be more intensely attacked by mustard than other portions of the cell.

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III. THE HISTOPATHOLOGY OF THE OCULAR LESIONS PRODUCED BY THE SULFUR AND NITROGEN MUSTARDS*

ALFRED E. MAUMENEE AND ROY O. SCHOLZ

The correlation of histopathological with clinical observation on the ocular lesions produced by the mustard group of agents have been published by a number of authors (1). The present study was undertaken in order to provide an adequate base for the more detailed and specialized studies, reports of which are to follow. It is presented here as a background to those further studies, and because a number of the histopathological observations which this study revealed have not been noted by the earlier investigators cited above.

For the purpose of the present study, doses of the toxic agents were used which cause severe ocular damage. When the lesion was allowed to run its full course following these doses, permanent corneal opacities resulted in almost all eyes, perforation of the cornea in 10-20%. Injuries of this severity by sulfur mustard were produced either by exposing the eye to saturated mustard vapor at 24°C. for 60 seconds or by placing a droplet of 140 micrograms of liquid mustard (Levinstein redistilled 95% pure) on the corneal limbus. Three nitrogen mustards were used for comparison: HN1 or $\text{H}_5\text{C}_2\text{N}(\text{C}_2\text{H}_4\text{Cl})_2$, HN2 or $\text{H}_3\text{CN}(\text{C}_2\text{H}_4\text{Cl})_2$ and HN3 or $\text{N}(\text{C}_2\text{H}_4\text{Cl})_3$. Most of the nitrogen mustard injuries were produced by placing a 3.5 cu. mm. droplet of a 5% aqueous solution of the HCl salt of the agent on the center of the cornea. In one series the eyes were exposed to saturated vapor of the free base of HN2 at 24°C. for 30 seconds. For the vapor exposures the chamber shown in Fig. 1 of the preceding paper (2) was used. The animal's eye was proptosed from its socket before the agent was applied and the lids allowed to close immediately afterwards. The eyes were removed at stated intervals after exposure, fixed in Zenker's solution, sectioned in paraffin and stained with hematoxylin and eosin. Rabbits were used for all of the observations to be reported in this paper.

* The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

OBSERVATIONS

All four toxic agents produced, with these dosages, lesions of approximately the same severity and similar in their histological characteristics. The lesions produced by all four agents will, therefore, be described together with only an occasional need to refer separately to the different agents.

Conjunctiva: The conjunctival damage resulting from exposure to the mustard agents shows a remarkable degree of uniformity. HN2

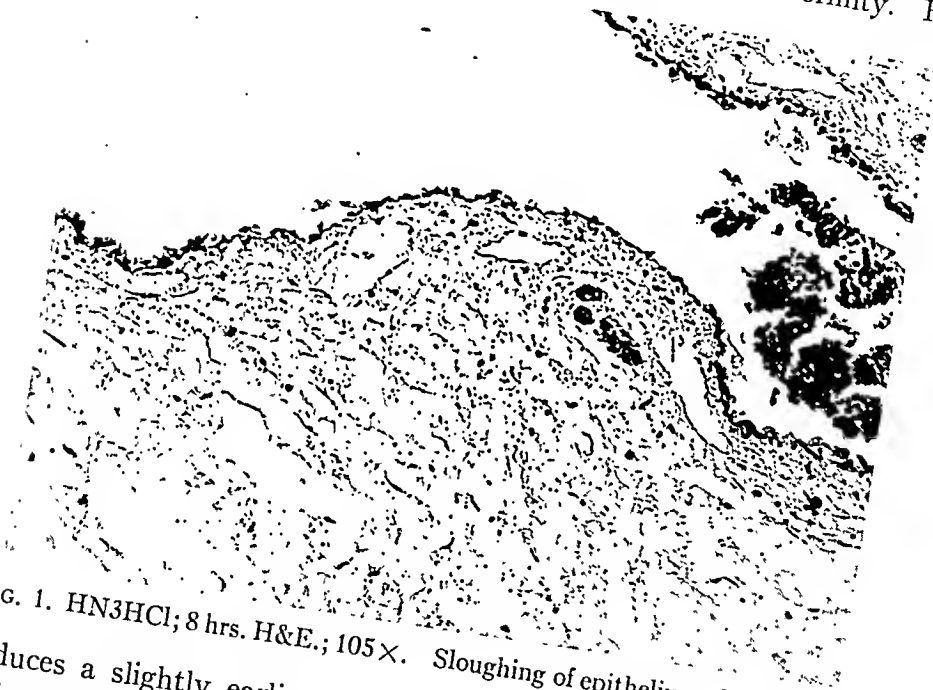


FIG. 1. HN3HCl; 8 hrs. H&E.; 105 \times . Sloughing of epithelium of conjunctiva.

produces a slightly earlier necrosis of the epithelium and more occlusion of the vessels of the conjunctiva than the other agents. HN1 causes the least severe damage. The number of hemorrhages in the tissue is greatest in HN2. This difference is more obvious on clinical than on histological examination.

Epithelium: The first change noted in the epithelium is a loss of the mucus from the goblet cells. This occurs about 30 minutes after exposure. An exudation of inflammatory cells appears on the conjunctival surface about 24 hours after the injury and continues until the 7th to 10th day. Beginning 8-24 hours after the exposure many

cells in the conjunctival epithelium become pycnotic and loosened from adjacent cells, and slough off (Fig. 1). As this process continues large areas become denuded of epithelium. Regeneration of the epithelium begins on about the 3rd or 4th day and is similar to that seen in the corneal epithelium. At first there is a single layer of irregular large flat cells which migrate into the denuded area, usually arising from crypts or folds that have escaped injury (Fig. 2). During the following week, the epithelium assumes a normal multi-layered



FIG. 2. HN₂HCl; 3 days. H&E.; 67 \times . Regeneration of conjunctival epithelium.

appearance with goblet cells. Even after this occurs, inflammatory cells can be seen passing through the epithelium if the reaction is severe.

Connective Tissue: The first observable effect of the local applications of these agents is a dilatation of the conjunctival vessels. Edema of the conjunctiva is seen in all cases by the end of 15 minutes and becomes steadily more prominent during the first 24 hours. This edema is slightly more noticeable in the early stages after nitrogen mustards than after sulfur mustard. Trypan blue injected intra-

venously during the first half hour after exposure escapes rapidly from the injured conjunctival vessels. During the next two days the interstitial fluid becomes more serous and by the 4th to 5th day it begins to be absorbed. Polymorphonuclear cells begin to infiltrate the connective tissue at 10-18 hours and increase to the 3rd to 7th day, after which they decrease as the general reaction of the eye subsides.

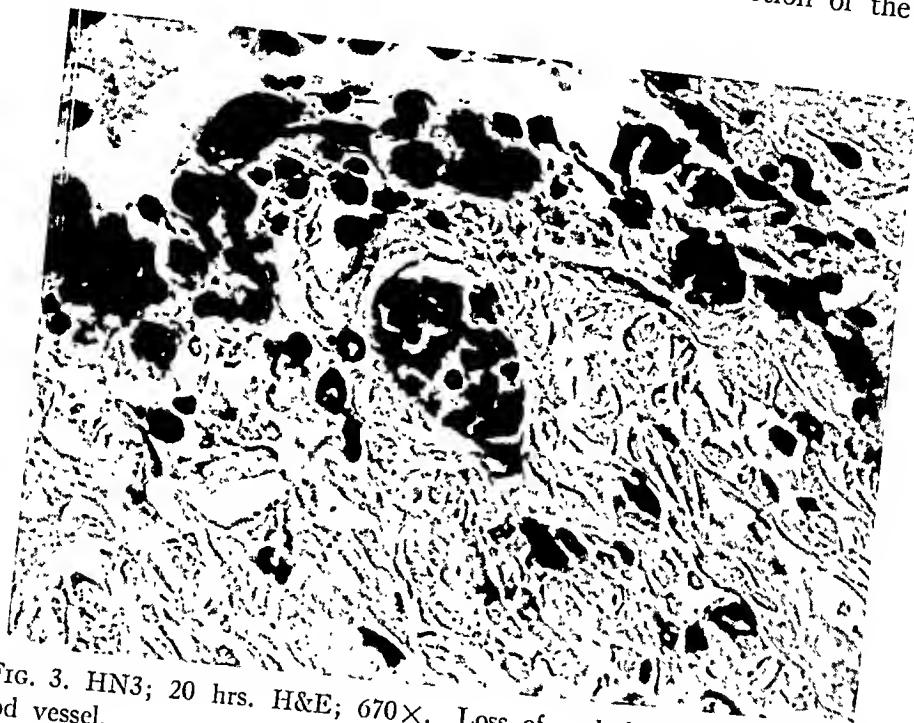


FIG. 3. HN3; 20 hrs. H&E; 670 \times . Loss of endothelium in conjunctival blood vessel.

Loss of the endothelium of the smaller vessels (Fig. 3) with subsequent occlusion begins at about 20-24 hours. The occluded vessels show densely packed red blood cells but no fibrin clot. At about the same time numerous small hemorrhages occur in the connective tissue. The larger blood vessels show less damage and remain unoccluded in most cases.

In spite of the early necrosis of the epithelium, occlusion of the blood vessels, edema, hemorrhages, and polymorphonuclear infiltration of the conjunctiva there is relatively little residual damage. Occasionally the conjunctival connective tissue becomes thickened and

more fibrous than normal as the acute reaction subsides. Formation of granulation tissue is extremely rare and extensive symblepharon is never found. Occasionally areas devoid of vessels or cells, corresponding to the clinically observed "pearly white" lesions in the conjunctiva, are seen. These lesions are seen as early as the first week after injury and such regions may remain devoid of cells for as long as two years. In the later specimens there is an overgrowth of capillaries in the tissue adjacent to these acellular areas. Larger vessels grow across

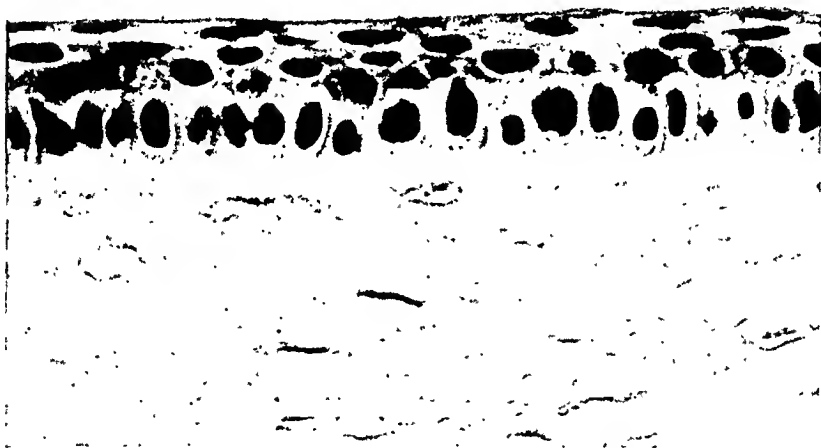


FIG. 4. H&E; 670 \times . Normal corneal epithelium.

the damaged tissue causing the "marbled" appearance of the conjunctiva.

There is usually slight damage to the nictitating membrane except for necrosis of the epithelium on the surface next to the globe. The large glands on the external surface occasionally lose their mucus and sometimes show squamous metaplasia. The cartilage of the nictitating membrane shows very little change.

Cornea: Epithelium: The sequence of pathological changes in the corneal epithelium following exposure to the mustard agents differs somewhat from that in the conjunctiva. Furthermore, the reaction

is slower in the corneal epithelium and because of this the various steps in the process can be followed in greater detail. The epithelial layer becomes loosened from the stroma a few hours after the injury at a time when many of the cells do not appear histologically to be dead. The time of appearance of the first histological evidence of injury to the cells and the subsequent changes that occur are about the same for all four agents studied.

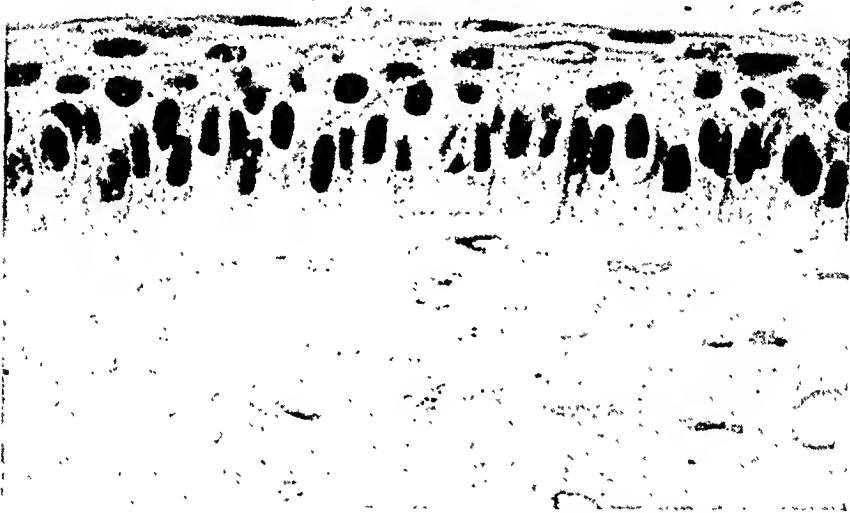


FIG. 5. HN3; 1 hr. H&E; 670 \times . Migration of basal nuclei of corneal epithelium with cystic changes in basal cells.

The first evidence of damage appears about 10 minutes after a burn. The nuclei of the basal columnar cells move from their normal position at the base of the cell to the more central portion of the cell. At the same time vacuoles appear in the cytoplasm near the nuclei in some cells, while in others the basal portion of the cytoplasm stains less intensely than normally, suggesting an imbibition of water (Fig. 5). The nuclei of the superficial cells, which are normally flat and take a dense homogeneous stain, become swollen so that they are oval, more lightly stained and appear granular. Shortly after this the nuclei of the basal cells begin to swell so that they no longer lie in a straight line

but overlap one another in the sections (Fig. 6). In some specimens about 2 hours after a mild injury scattered nuclei in the basal layers of the epithelium begin to show changes which have been described as fragmentation, and which are discussed in greater detail in one of the succeeding papers (3). The nuclear chromatin first forms intensely stained irregular masses within the nucleus. The nuclear membrane then disappears and the chromatin fragments are dispersed throughout

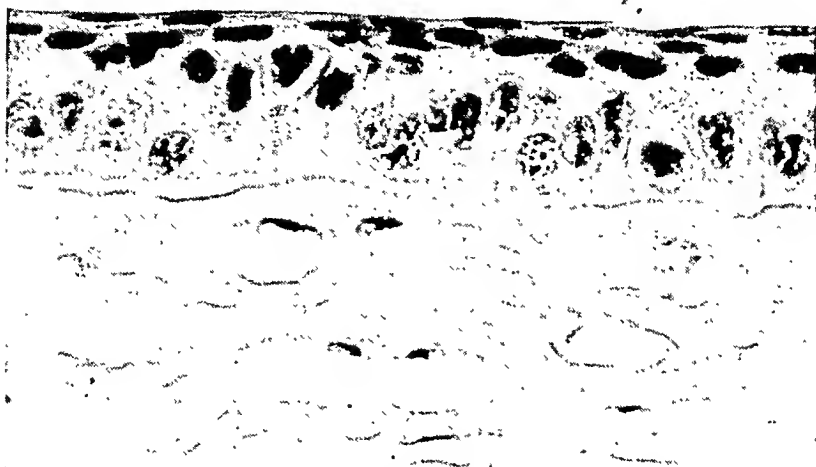


FIG. 6. HN3HCl; 12 hrs. H&E; 670 \times . Swelling of nuclei in corneal epithelium.

the cell (Fig. 7). Twenty-four hours after a more severe injury a larger number of pycnotic cells can be seen scattered through the epithelium. This is true for injuries by vapor and by the aqueous solutions of nitrogen mustard HCl salts. A slight difference has been noted, however, in eyes injured by a droplet of liquid mustard. In these eyes, a localized area of uniformly pycnotic cells can be seen where the droplet rested (Fig. 8). The adjacent epithelial cells show the changes that have been described above.

After the first 4 hours, the epithelium begins to become detached from the stroma. The line of separation between the epithelial cells



FIG. 7. Mustard vapor; 4 hrs. H&E; 670X. Epithelial nuclear fragmentation.



FIG. 8. Mustard Splash; 12 hrs. H&E; 670X. Pycnotic cells in area of splash exposure.

and the stroma occurs either between the epithelial cell membrane and the stroma or just inside of the cell membrane, leaving the basal part of the cell attached to the stroma (Fig. 9). The detachments vary from eye to eye but in general become more marked until 24 to 36 hours at which time most of the epithelium has sloughed off. The phenomenon of epithelial loosening is discussed in greater detail in some of the succeeding papers (4).

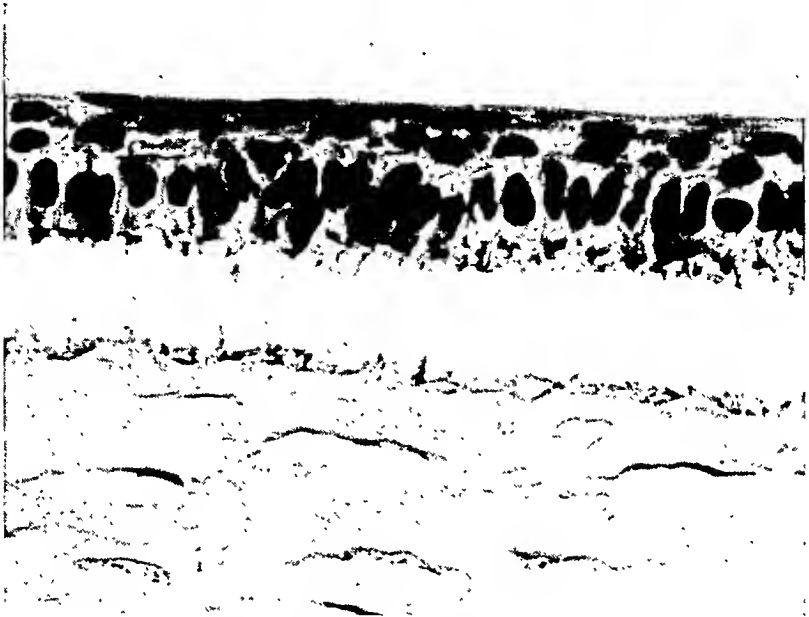


FIG. 9. HN3; 8 hrs. H&E; 670 \times . Separation of epithelium from stroma.

The time of regeneration of the corneal epithelium varies according to the severity of the injury. The cells are probably derived from two sources: (1) migration of the remaining corneal epithelial cells, and (2) a sliding of the limbal conjunctival epithelium over the cornea. The latter type of repair was first described by Mann and Pullinger (1g) and is frequently seen in mild injuries. Between 24–48 hours the pigmented cells of the limbus begin to move en mass across the cornea forming a grossly visible line (Fig. 10). Frequently epithelium of conjunctival type with goblet cells can be seen on histological examination between this line and the limbus for a few days. These



FIG. 10. Mustard Splash; 4 days. H&E; 120 \times . Migration of pigment line. 4 mm. from limbus.



FIG. 11. HN3; 4 days. H&E; 670 \times . Regeneration of corneal epithelium.

cells later may be transformed into normal corneal epithelium or displaced by regenerating corneal epithelium, and the pigment line disappears from the cornea in a few weeks. In the more severe injuries epithelial regeneration begins on the third or fourth day by a migration of a single or double layer of cells. These cells are large, flat, take a pale stain, and have gaps in their cytoplasm and between the cells. The gaps are probably artifacts due to fixation, but they show that the cells do not have their normal consistency (Fig. 11)

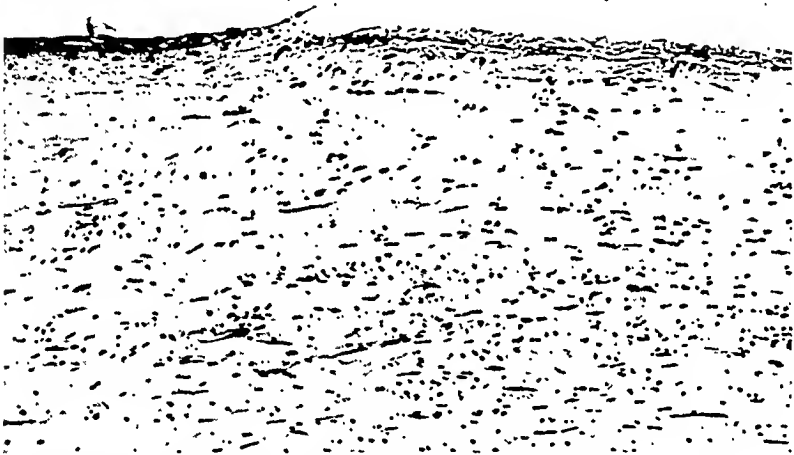


FIG. 12. Mustard Splash; 6 mos. H&E; 150 \times . Loss of epithelium over heavily scarred and infiltrated corneal stroma.

This thin irregular layer of epithelium usually develops into apparently normal corneal epithelium in a few days if the underlying stroma is normal. However, if the superficial stroma remains edematous or infiltrated with polymorphonuclear cells, the epithelium continues as a thin irregular layer as in the first steps of regeneration and is easily detached from the stroma. This incomplete regeneration resulting, perhaps, from recurrent erosion, has been noted as long as 6 months after a mustard injury (Fig. 12). Frequently in eyes removed 2–6 months after exposure to mustard, the epithelium over heavily vas-

cularized cornea is irregularly thickened with 7 to 8 layers of cells and peg-like processes extending into the stroma (Fig. 13). Occasionally the conjunctival epithelial cells which have migrated over the vascularized stroma fail to be transformed into corneal type of epithelium and retain their normal number of goblet cells. At the point where the vascularization of the cornea stops there is then an abrupt change from the conjunctival type of epithelium to the corneal type (see Fig. 4 of succeeding paper).

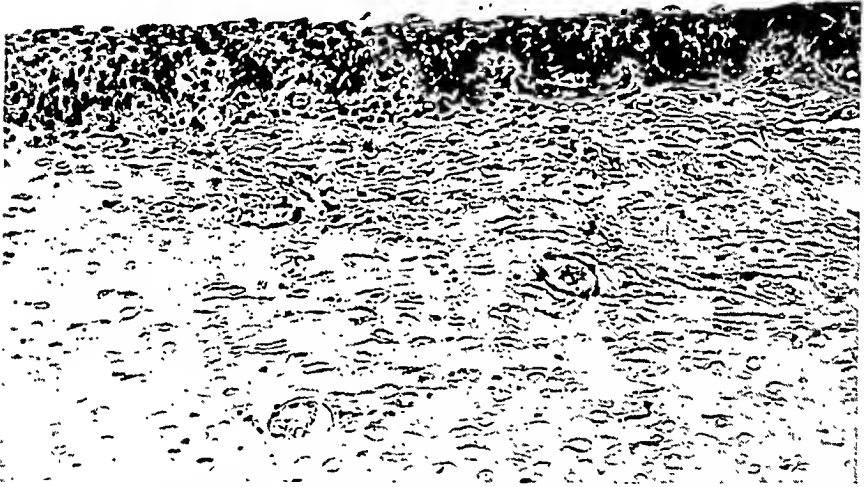


FIG. 13. HN1; 5½ mos. H&E; 670X. Thickened epithelium over scarred corneal stroma.

The diameter of the regenerating cell varies up to 30 micra as compared to 10 micra of the normal basal epithelial cell. Thus one regenerating cell may cover 9 times the area previously covered by one cell. Since the migrating cells are only one to two layers thick as compared to four to five cells in the normal epithelium, a remainder of 5–10% of the cells can cover the denuded area caused by the injury.

Stroma: The appearance of the histological damage that occurs in the stroma is also very similar after exposure to any one of the four agents studied. Corneal edema of the "primary type" described by Mann and Pullinger (1f) appears at the end of the first hour in the

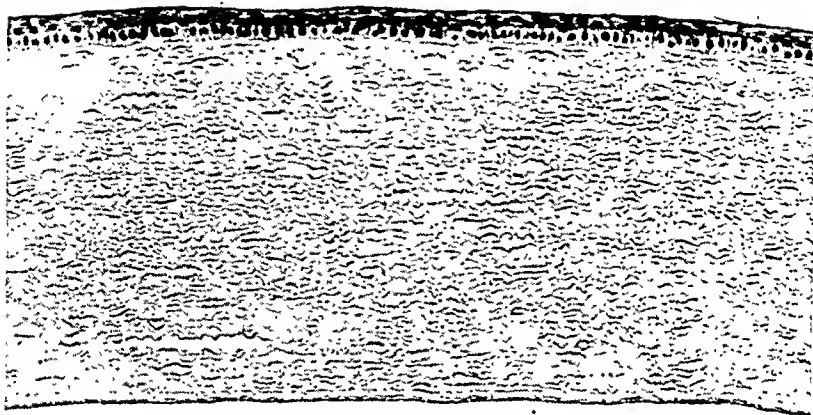


FIG. 14. H&E; 150 \times . Normal cornea.

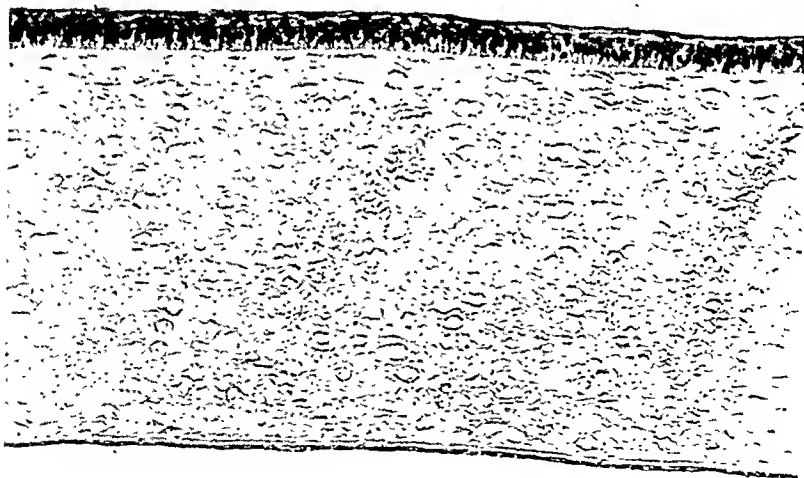


FIG. 15. HN3; 1 hr. H&E; 150 \times . Edema in posterior part of corneal stroma.

posterior layers of the cornea (Figs. 15, 16). This edema is probably due to an increased permeability of the endothelial cells although they

show no histological evidence of damage at the time. In this respect it is similar to the edema of the conjunctiva which likewise appears before visible histopathological changes in the capillary endothelium. "Primary edema" continues to increase for 24 to 48 hours and the corneal thickness may become two to three times as great as normal. Polymorphonuclear cells begin to invade the anterior two-thirds of the cornea at the end of 24 to 48 hours and continue to increase in numbers until the 3rd to 7th day. The number of invading cells

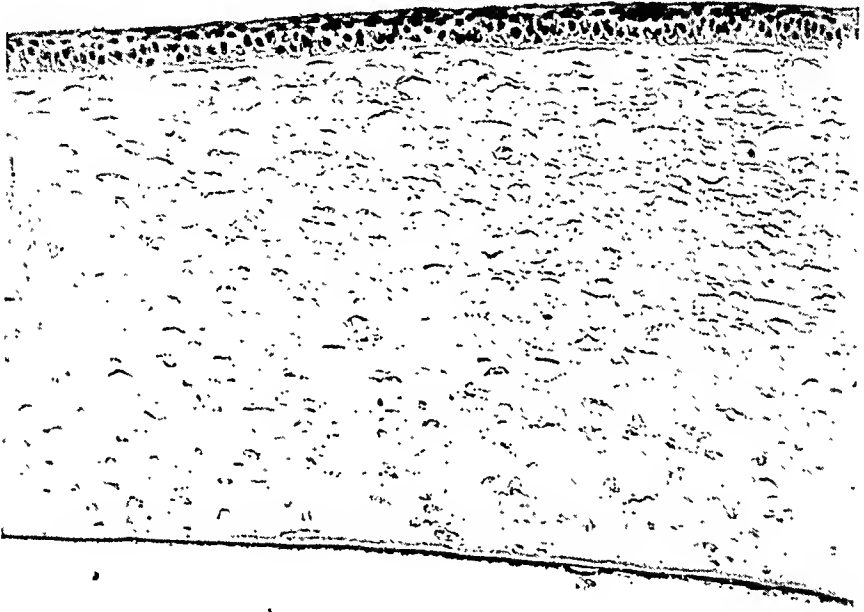


FIG. 16. HN3; 8 hrs. H&E; 150 \times . Edema of corneal stroma.

depends to a large extent on the number of destroyed corneal corpuscles and also on the intensity of the secondary infection that almost always occurs. As the acute reaction begins to subside on the 7th to 8th day, monocytes can be seen among the inflammatory cells. Pullinger and Mann (5) have suggested that some of the invading macrophages may eventually become fibroblasts and aid in the repair of the stroma. The possibility of such a transformation of macrophages to fibroblasts has been demonstrated by Ebert and Florey (6). At about 4-5 days, blood vessels and fibroblasts also begin to invade the stroma from the limbus and as time advances, areas of typical

granulation tissue may replace the destroyed stroma tissue (Fig. 17). The invasion of the blood vessels and fibroblasts is usually limited to the anterior two-thirds of the stroma. In the posterior part of the stroma many corneal corpuscles usually survive the injury (Figs. 18, 19).

The pathological changes that occur in the stroma cells are more difficult to observe than the changes that occur in the epithelial cells, for even in the normal rabbit's cornea the outline of the stroma cells

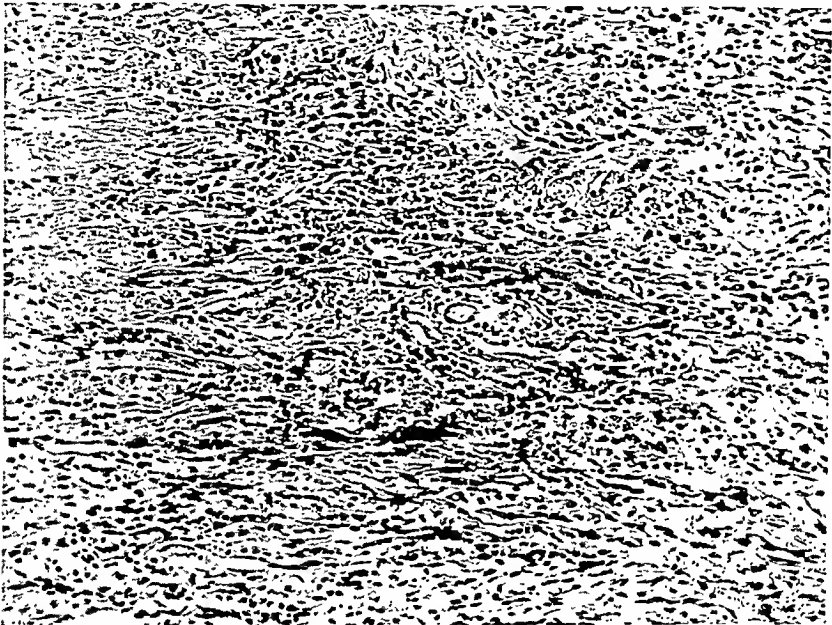


FIG. 17. HN3; 5 days. H&E, 150 \times . Granulation tissue in corneal stroma.

cannot be seen and the nuclei are thin homogeneous dark blue staining streaks (Fig. 14). Degenerative changes can be seen in the cells of the anterior two-thirds of the cornea, however, as early as 8 hours after a burn. The changes appear either as a clumping of the chromatin material so that the nuclei look like a bit of string with three to four knots tied in it, or the nuclei may swell and disintegrate. In the material studied for this report more cells showed the pycnotic type of degeneration after mustard and more became swollen and disintegrated after HN2. By the end of 24-48 hours most of the cells in the anterior stroma showed one or the other of these changes or had

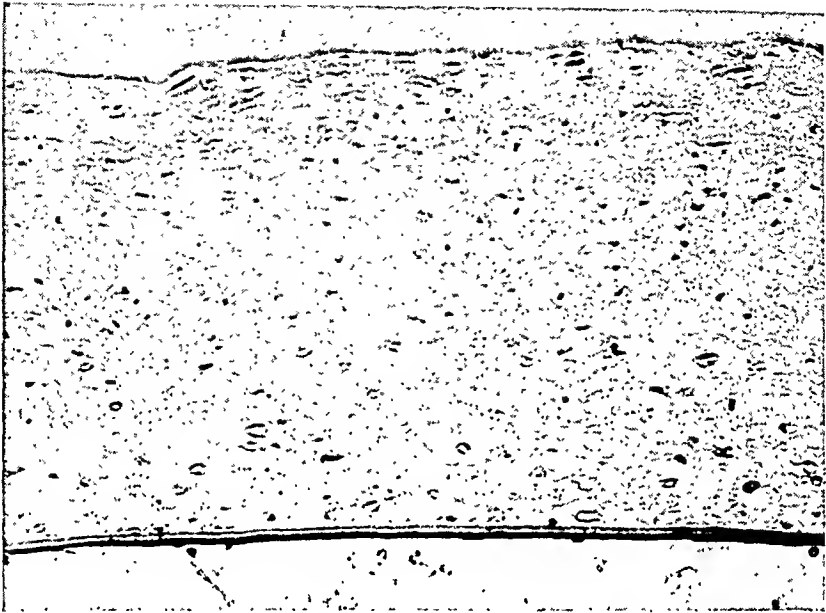


FIG. 18. Mustard Vapor; 24 hrs. H&E; 150 \times . Pycnosis of cells in anterior stroma and swelling of cells in posterior part of corneal stroma.

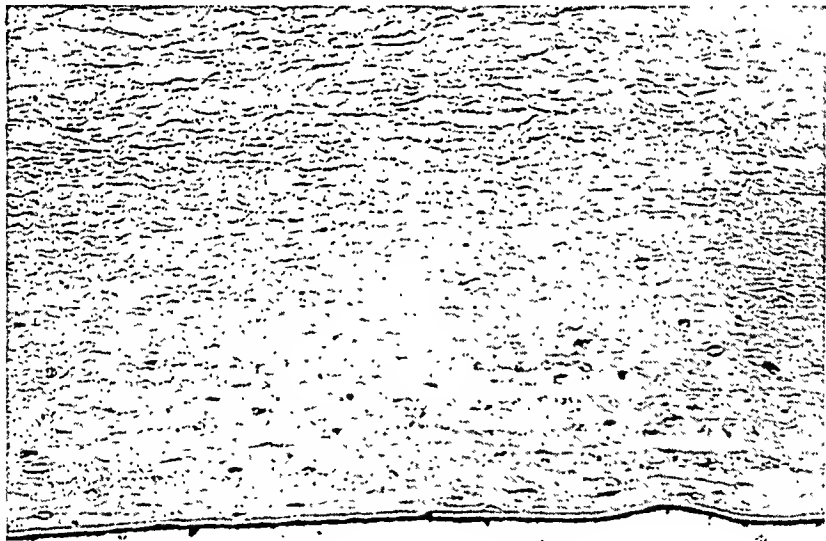


FIG. 19. HN1HCl; 2 days. H&E; 150 \times . Absence of cells in anterior stroma and swelling of cells in posterior stroma of cornea.

disappeared completely from the tissue (Figs. 18, 19). The cells in the posterior third of the stroma do not appear entirely normal, but

some survive in a high percentage of the eyes studied. If corneal edema is produced by a mechanical injury to Descemet's membrane without applying any vesicant agent, the corneal cells swell, become more clearly outlined and resemble fibroblasts (Fig. 20). During the edema that follows mustard or nitrogen mustard, the corneal cells that remain in the posterior third of the stroma, however, seldom show this picture but have markedly swollen, pale, granular staining nuclei with some margination of the chromatin, indicating that some

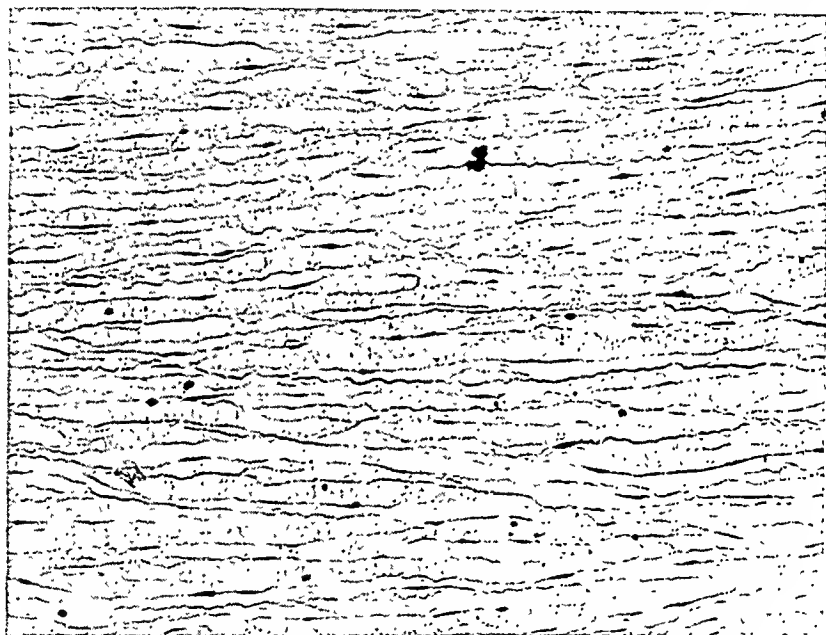


FIG. 20. H&E; 150 \times . Edema of stroma following mechanical injury to Descemet's membrane.

damage has been done to them other than that produced by edema alone (Fig. 19). These changes have been studied in greater detail and are reported in a succeeding paper (7).

Replacement of damaged stroma cells takes place slowly. The conjunctiva appears almost normal in one to two weeks but vascularization and replacement of the cells in the anterior two-thirds of the cornea is usually just beginning at this time. The inflammatory reaction does not subside nor the cornea return to its normal thickness for two to three months.

In some eyes, blisters of serum containing a few polymorphonuclear and mononuclear cells are found under the epithelium for as long as 3-6 months after injury (Figs. 21, 22). The underlying stroma in these areas is usually heavily vascularized and contains many polymorphonuclear cells. The epithelium is loosely attached and may easily slough to form an ulcer. These areas are probably one of the causes of recurrent ulcerations of the cornea following mustard injury. Long after primary healing, in some eyes in which the cornea has regained complete or almost complete normal clarity areas may be

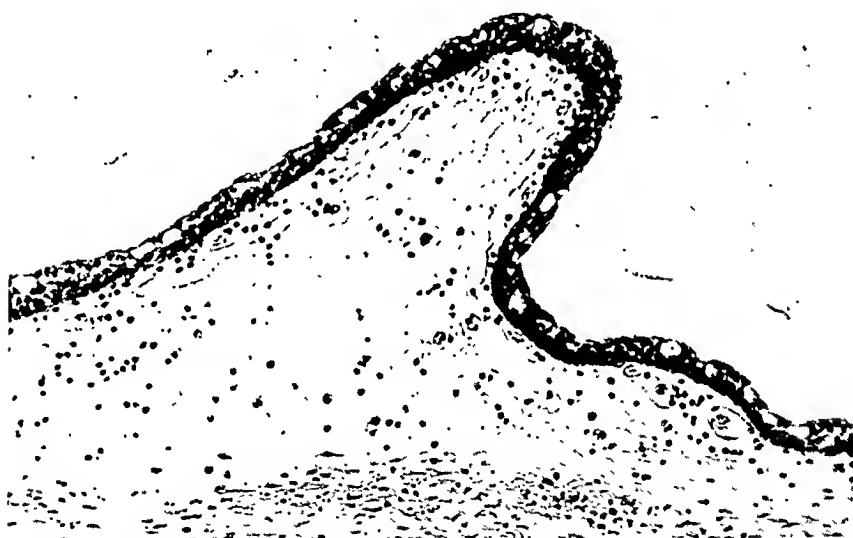


FIG. 21. HN3HCl; 4 mos. H&E; 150 \times . Edematous bleb under corneal epithelium.

found in the stroma that are still completely devoid of cells (Fig. 23). These acellular areas may be as much as 2-3 mm. in diameter and $\frac{1}{3}$ to $\frac{1}{2}$ the thickness of the cornea. It is possible that these lesions are analogous to the "pearly white" lesions in the conjunctiva in that the fixed tissue cells are destroyed and are not replaced either by regeneration or by scar formation. It seems likely that these acellular areas may also account for some of the late recurrent ulcers. Accumulations of cholesterol crystals as described by Mann and Pullinger (1f) have only occasionally been seen in our specimens and

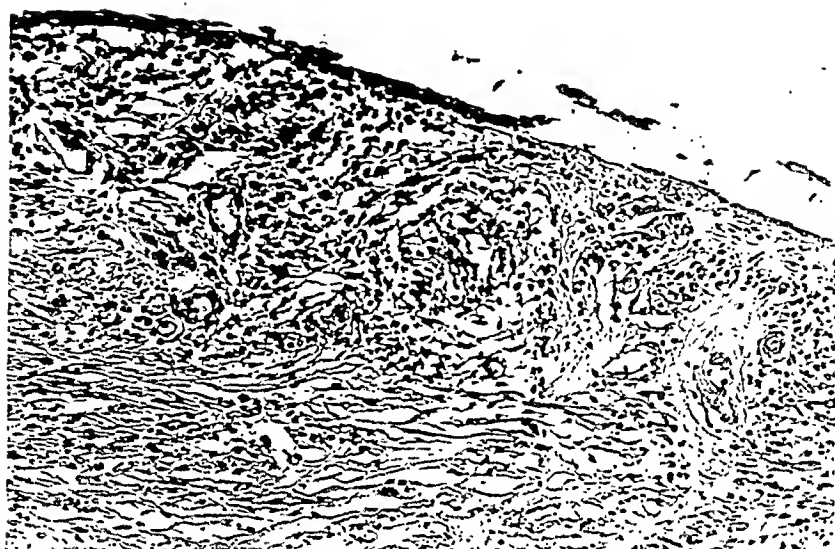


FIG. 22. HN1; $3\frac{1}{2}$ mos. H&E; $150\times$. Inflammatory reaction in scarred corneal stroma with loss of epithelium.

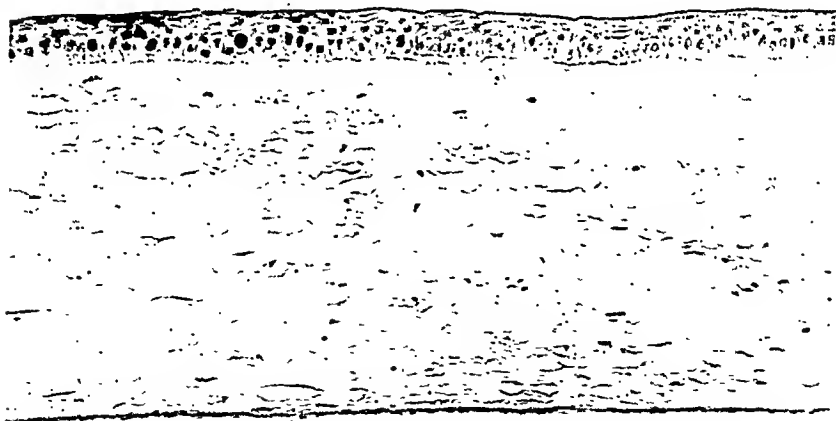


FIG. 23. HN2 vapor; Treated with sodium diethyldithiocarbamate; 21 days. $150\times$. Clinically clear cornea. Marked reduction in number of stromal cells.

the epithelium over these areas appeared normal. Mann and Pullinger attribute late ulcerations to the presence of these crystals but we have been unable to correlate recurrent erosions with the presence of these crystals.

During the first few days after the application of any one of these toxic agents, the clinical symptoms are dominated by the reactions which occur in the conjunctiva. During this period the severity of the reaction to a particular dose of the agent is remarkably uniform and reproducible from rabbit to rabbit. The corneal symptoms that occur during this period, loss of the epithelial and endothelial coverings, primary edema, and beginning polymorphonuclear infiltration are also relatively uniform. After the 3-4 day, however, as the conjunctival lesions begin to recede, increasing variability in the course of the reaction appears. The same dose that leads to perforation of the cornea in some eyes may result in little or no final corneal opacity in others. This variability in the ocular reaction to mustard is much greater than that found after exposure to some other toxic agents, for instance lewisite. It seems clear that most of this variation concerns events which take place in the corneal stroma.

Part of this variation can be attributed to differences in the intensity of secondary infection, but this by no means accounts for the extent of the scatter. Maumenee and Guyton (8) studied a large series of rats all exposed to as nearly as possible the same dose of mustard and treated with antibiotics. In spite of the fact that the conjunctival bacterial flora was kept at minimal levels throughout the experiment in these eyes, the severity of the reaction varied almost as widely in these animals as in those not treated with antibiotics.

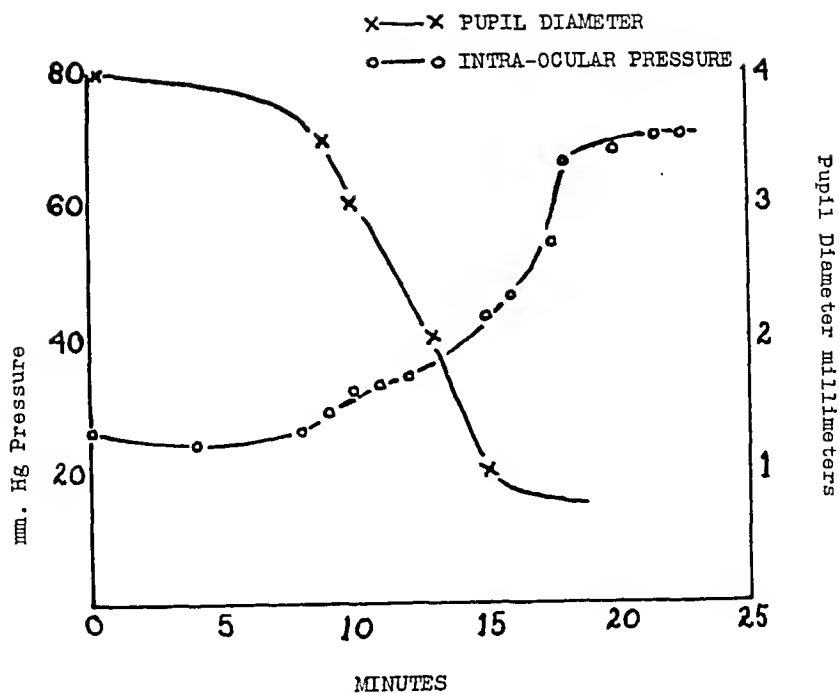
One factor which appears to be correlated with this variation is the variation in the number of dead corneal stroma cells. The intensity of polymorphonuclear infiltration in the cornea, which reaches a maximum about one week after the injury, is closely connected with the number of dead cells. There is, moreover, a close correlation between the intensity of polymorphonuclear infiltration and the liquefaction and disorganization of the corneal lamellae which develops during the second and third weeks. Finally, residual scarring of the cornea is greatly increased if the lamellae have become disorganized so that invading fibroblasts and regenerating keratoblasts are not laid down

in parallel rows. Wide variation in the number of lost or fatally injured cells in the corneal stroma can be recognized in sections as early as the second or third day after exposure.

Endothelium: In contrast to the wide variation in injury to the corneal stroma cells, for a given dose of a given one of these toxic agents, the reactions in the endothelium are much more uniform, but there is a wide variation in relation to variations in dosage and in respect to the four different toxic agents that are being compared. With doses that produce approximately equal reactions in the conjunctiva and corneal stroma, the effect on the endothelium is most to least marked with the agents in the order $\text{HN2} > \text{HN3} = \text{Mustard} > \text{HN1}$. After small doses the endothelium shows very little histological change, but some damage must occur as evidenced by the increased permeability of the cells resulting in corneal edema. In all injuries with the standard doses the edema of the posterior cornea develops within 8-10 hours after the injury, before there is a sloughing of the endothelium. With HN2 the edema begins as early as one hour after exposure. Sloughing of the endothelium after exposure to HN2 is first seen about 8-24 hours after exposure. Similar changes appear as late as 3 to 4 days after HN1. The other two agents fall between these extremes. Fragmentation of the nuclei of the endothelial cells has been observed in supravital preparations (3) but was not noted in this study. Regeneration of the endothelium is markedly dependent on the depth and severity of the injury. It is very much slower than the regeneration of the epithelium and begins about 10-14 days after the injury. A marked inflammatory reaction in the anterior chamber with wandering cell deposits on Descemet's membrane seems to retard this regeneration.

Aqueous: An increase in the protein content of the aqueous occurs early in all cases but is more marked with HN2. The cellular reactions depend on the dosage of the agent and extent of damage to the cornea. Strangely, there are only slightly more cells in the aqueous after HN2 than after the other agents.

Iris and Ciliary Body: In the iris again HN2 produces by far the greatest damage. The pupil constricts rapidly following application of this agent. Coincidental with this constriction the intraocular pressure rises (Graph 1). The earliest damage visible histologically



GRAPH 1. PUPILLARY DIAMETER AND INTRAOCULAR PRESSURE FOLLOWING LOCAL APPLICATION OF HN2

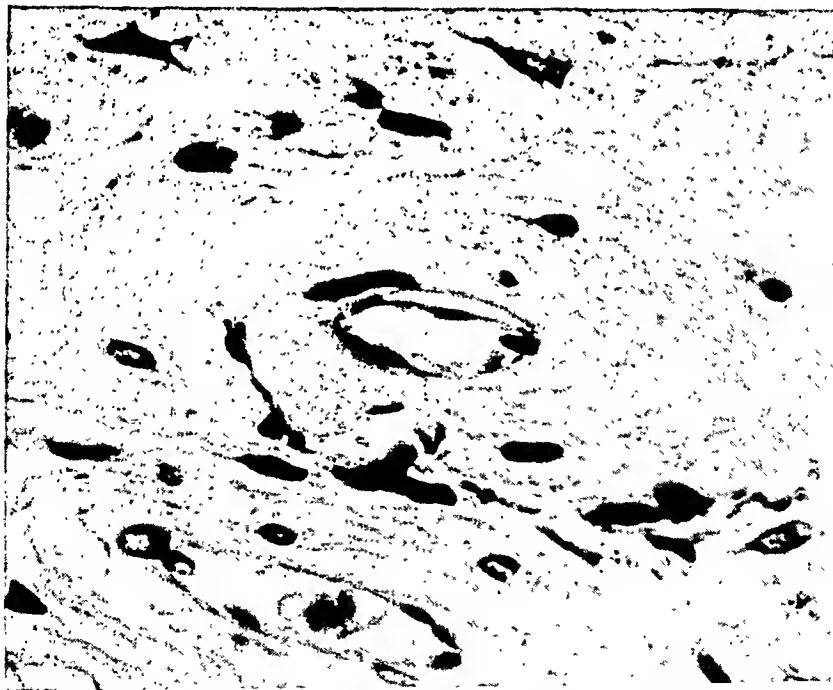


FIG. 24. HN2HCl; 3 hrs. H&E; 670X. Detachment of capillary endothelium in iris.

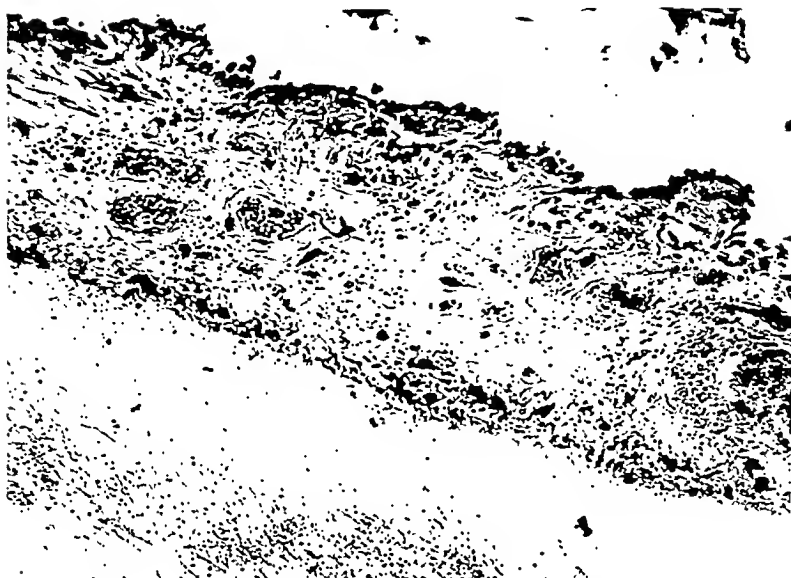


FIG. 25. HN2 Vapor; 4 days. H&E; 150 \times . Hemorrhages in necrotic iris.



FIG. 26. HN2 Vapor; 4 days. H&E; 150 \times . Hemorrhages and Greaf vesicles in ciliary processes.

in the iris is a detachment of the capillary endothelium (Fig. 24). The nuclei of the detached endothelium do not stain as intensely as

normal. Later the endothelium disappears and the capillaries become occluded by packed red blood cells without fibrin clots. Occlusion of the capillaries and hemorrhages occur as early as 8 hours. On the third or fourth day the iris usually shows extensive necrosis (Fig. 25). The other agents produce occasional small iris hemorrhages and desquamation of capillary endothelium. Mustard and HN1 show the least damage to the iris. All four agents produce Greef vesicles on the ciliary processes within one half to one hour after exposure. HN2 again produces the most marked changes in the ciliary processes (Fig. 26). Greef vesicles appear with 5 minutes after application of this agent.

Lens: Clinically a diffuse clouding of the lens can be observed several weeks after contamination with HN2. The lens was not examined histologically in this study.

DISCUSSION

Injury: The conjunctiva shows marked changes in the early stages of injury in the form of edema of the stroma, loss of the endothelium and occlusion of the small capillaries and necrosis of the epithelium. In the corneal epithelium the first changes to be noted are a fragmentation of some nuclei in the basal layer of cells and then a detachment of the epithelial cells from the underlying stroma. Later some nuclei became pycnotic and the epithelium sloughs off. The first manifestation of damage to the stroma is an edema of the posterior portion which occurs as early as one hour after injury in the HN2. This is probably due to an increase in the permeability of the endothelium. The nuclei of the stroma cells become pycnotic or swell and burst as early as eight hours after damage. Polymorphonuclear cells begin to invade the stroma at about 24 hours. The extent of this type of infiltration depends on the number of stroma cells which have been killed and on the intensity of the secondary infection present. The first histological evidence of damage to the endothelium of the cornea is a sloughing of the cells. The time of occurrence of these lesions varies considerably with the agent used. It occurs as early as 8 hours after HN2 and as late as 3-4 days after HN1. Damage to the iris is relatively mild after the application of HN1 or mustard. However, after HN2, and occasionally and to a lesser degree after HN3, there

occurs a loss of the endothelium of the capillaries with subsequent occlusion of the vessels and necrosis of the iris. Greef vesicles appear on the ciliary processes after the application of all four agents. In addition to these vesicles, hemorrhages occur in the ciliary processes after HN2.

Repair: The conjunctival epithelium is repaired by migration of the uninjured epithelial cells from adjacent areas. The edema is absorbed, and the necrotic debris is removed by the macrophages. New formed capillaries reestablish the circulation when there has been extensive damage to the blood vessels. Occasionally necrotic areas remain acellular over long periods of time in the regions where "pearly white" areas are observed clinically. Markedly dilated vessels can also be observed in some eyes. Extensive areas of granulative tissue and symblepharon are almost never found.

The areas denuded of corneal epithelium are covered by a sliding of adjacent uninjured corneal epithelial cells and by a migration or the conjunctival epithelial cells. The migration of the pigmented epithelium of the limbus across the cornea, and the presence of conjunctival goblet cells over the corneal stroma, is proof of the latter type of repair. If the stroma underlying the new epithelium is normal, the new cells will transform themselves into typical corneal epithelial tissue, or are replaced by corneal epithelium. If, however, the stroma under these new cells is edematous or is invaded by blood vessels, then the epithelium will either remain as a thin sheet sometimes preserving evidences of its conjunctival origin, or become thicker than normal, and may slough off frequently.

The repair of the corneal stroma is somewhat more complicated than the corneal epithelium or the conjunctiva. The mode of repair depends on the extent of the damage done. If a relatively mild lesion is produced in the center of the cornea a few polymorphonuclear cells will invade the stroma and remove the dead corneal cells. A few days later large wandering mononuclear cells, probably macrophages, are seen in the area of the lesions. The dead corneal cells are probably replaced by a transformation of these macrophages into keratoblast or by a migration of uninjured stromal cells. As the corneal edema subsides relatively little opacification will remain after this type of lesion. If a more severe injury is produced a greater number of

polymorphonuclear cells will invade the stroma and there will be a destruction of the corneal lamellae. A few days later blood vessels will begin to invade the stroma along with or just after the macrophages. Numerous fibroblasts are seen in the regions of the new vessels. The repair does not take place in an orderly fashion along the old corneal lamellae. Areas of typical granulation tissue are seen in some places and in other places areas of edema persist for a year or more. This type of repair always results in greater scarring than the previously mentioned type of repair. Some areas may remain acellular for a very long time.

Repair of the endothelium is markedly dependent on the depth and severity of the injury produced. It is very much slower than the epithelium and begins 10-14 days after injury. The endothelial cells are replaced by a migration of the uninjured cells.

Repair of the iris is essentially the same as that of the conjunctiva.

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IV. EFFECTS OF MUSTARD AND NITROGEN MUSTARD ON MITOTIC AND WOUND HEALING ACTIVITIES OF THE CORNEAL EPITHELIUM*

JONAS S. FRIEDENWALD, WILHELM BUSCHKE, AND ROY O. SCHOLZ

The data reported in a previous paper serve to indicate the complex intracellular distribution of some of the bound mustard. Between the initial reaction of mustard with the tissue, and the consequent death of the cells, there is a gap in our knowledge so great as almost to defy analysis. As a first step in the attack on this problem we have assumed that there are many different physiological mechanisms within the cell that are capable of being damaged by exposure to mustard, and have attempted to isolate for study those physiological activities that are experimentally approachable. If the inhibition or malfunction of some intracellular physiological mechanisms could be connected with cellular death, the gap between the biochemical events of the primary reaction of mustard with tissue and the pathological events of cellular death might be somewhat narrowed.

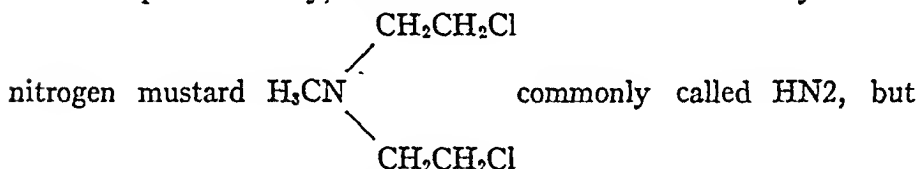
One normal activity of the cornea is the mitotic division of cells in the corneal epithelium. Recent studies in this laboratory (1) have shown that the rat's corneal epithelium is a favorable test object for the study of mitotic activity, and as a result of these studies convenient methods of assay were readily available. Some suggestion that mitotic activity might be particularly susceptible to inhibition or malfunction after exposure to mustard was implied in the report of Berenblum (2) that mustard acted as an antagonist to carcinogens on the rat's skin.

The techniques used for the assay of mitotic activity in the corneal epithelium have been reported elsewhere (1). The basic data which these studies revealed regarding the mitotic activity of the corneal epithelium of the rat are as follows: The corneal epithelium in animals of 50 to 150 gram weight contains approximately 2,000,000 cells, of which approximately 1,400,000 are in the two basal layers. Between 5,000 and 6,000 cells are normally found in mitosis. The duration of

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

the mitotic cycle of a single cell is about 70 minutes. The average duration of the resting stage between mitoses is 7-10 days. The mitoses occur exclusively in the two basal layers and are slightly more numerous near the periphery of the cornea than at the center. Two drugs which have been found most useful in analyzing the mitotic activity are colchicine and ether. Colchicine causes an arrest of mitosis in metaphase and hence an accumulation of mitoses with the passage of time. In proper dosage it apparently has no effect on the rate at which cells enter mitosis. It may be used, therefore, to determine the number of cells entering mitosis during a given period. Brief ether anesthesia stops the entrance of cells into mitosis for a period of 1 to 2 hours but does not, in proper dosage, influence the progress of cells through the mitotic cycle once mitosis has begun. It may be used, therefore, to determine whether the progress of cells through the mitotic cycle is normal or delayed.

In the present study, attention has been focussed chiefly on the



it may be said in advance that qualitatively similar results have been obtained with mustard and with other nitrogen mustards.

EFFECT OF LOCAL ADMINISTRATION OF HN2 HYDROCHLORIDE

In a preliminary study it was found that the local toxic effects of freshly prepared solutions of HN2 hydrochloride instilled in the eye were indistinguishable from those produced by exposure to the vapor of the free base. In the present study aqueous solutions were used unless otherwise specified. The solutions were either freshly prepared or freshly diluted from a 1% stock solution of HN2 hydrochloride in M/1000 HCl. The stock solution was kept on ice and renewed every two weeks. A single drop (approximately 0.05 ml.) of the solution was instilled into one eye of a 50-150 gram rat and the lids held open for 30 seconds after the instillation. At various intervals after the instillation the animals were sacrificed and the number of mitoses counted in the treated and untreated corneas. Counts were made on

a meridional strip of each cornea constituting $\frac{1}{30}$ of the total area of the cornea. The average normal number of mitoses in such a strip is 100-120. Since there is considerable individual variation in mitosis counts on different rats, but little normal variation between the two eyes of the same rat, the numbers given in Table I are the ratio of mitoses in the treated versus the untreated eye of the same rat. In general, the number given is the average of two or more animals for each observation.

TABLE I
Per Cent of Mitosis in Treated Versus Control Eyes

CONCENTRATION OF HN2 HYDROCHLORID	TIME AFTER ADMINISTRATION			NUCLEAR FRAGMENTS	LOOSENING OF EPITHELIUM
	3 hrs.	6 hrs.	24 hrs.		
.25%	43	2	0	+	+
.125	9	0	0	+	+
.06	8	8	0	+	±
.03	4	0	0	+	0
.015	15	43	0	+	0
.0075	24	20	10	+	0
.0037	32	35	6	+	0
.0175		38	33	0	0
.0008		36	52	0	0
.0004		53	52	0	0
.0002			84	0	0
.00005			134	0	0
.000012			147	0	0
.000003			110	0	0

Thresholds: A single drop of 1% HN2 hydrochloride produces a severe lesion in a rat's eye. 0.5% produces a slight lesion. With 0.25% few symptoms are clinically visible; however, histological study reveals during the first 24 hours areas of loosening of the corneal epithelium and some leucocytic infiltration of the tissue. At a concentration of 0.06%, no changes whatever are noted on routine histological study. 0.1% therefore, represents the threshold of local necrotizing action. In human beings pain, lacrimation, and photophobia, associated with fine punctate opacities in the epithelial layer of the cornea, are seen with exposures insufficient to elicit such severe clinical symptoms as those which were observable in the rat.

The administration of HN2 hydrochloride in concentrations between 0.25% and 0.0004% results in a marked decrease in the number of mitoses in the corneal epithelium. The onset and duration of this period of decreased mitosis varies with the dosage. At still lower concentrations there is a slight increase in the number of mitoses in the treated as compared with the untreated eye. A comparison of the mitosis inhibition produced by various toxic agents is given in Table II. The absolute amounts of these substances administered is of no special significance since the toxic agents were dissolved in

TABLE II
% Mitosis Inhibition 24 Hours after Administration

SUBSTANCE CONC. %	HN1	HN2	HN3	MUSTARD	LEWISITE
0.25		100	100		90*
0.12		100		*	42
0.06	*	100*	100*	98	34
0.03		100			15
0.015		100	100		
0.008		90		99	
0.004	87	94	85	93	
0.002	3	67	73	74	
0.001		48		25	
0.0005		48			

HN1 = $\text{H}_4\text{C}_2\text{N}(\text{C}_2\text{H}_4\text{Cl})_2\text{HCl}$ dissolved in water.

HN2 = $\text{H}_2\text{CN}(\text{C}_2\text{H}_4\text{Cl})_2\text{HCl}$ dissolved in water.

HN3 = $\text{N}(\text{C}_2\text{H}_4\text{Cl})_2\text{HCl}$ dissolved in water.

Mustard = $\text{S}(\text{C}_2\text{H}_4\text{Cl})_2$ dissolved in hexane.

Lewisite = $(\text{ClCH}=\text{CH})\text{AsCl}_2$ dissolved in triacetin.

* = threshold concentration for clinically visible effects.

different solvents and the amount taken up by the cornea no doubt varies with different agents and different solvents. For comparison between the different agents the relation between the threshold dose for the production of clinically visible symptoms and the threshold dose for inhibition of mitosis seems more significant. These thresholds are indicated in the table. It is evident that mustard and the three nitrogen mustards studied produce essentially similar effects, the threshold doses for mitosis inhibition with these agents being about 1/100 the threshold dose for clinically recognizable symptoms.

Fragmented Nuclei: Returning to the HN2 series which was studied in greater detail, it was found that while normal mitoses are almost completely absent in many of these preparations, a number of them show scattered cells in which the chromatin is fragmented and the cell disintegrating. The appearance of these cells closely resembles similarly disintegrating cells seen after colchicine poisoning. Occasionally a few cells with this type of disintegration are found even in normal controls. They are found almost exclusively in the basal layers of the epithelium. The number of these disintegrating cells is generally relatively small. This phenomenon of nuclear fragmentation and cellular death produced by mustard and nitrogen mustard injury has been studied in some detail. The results of these studies are reported in the next paper.

ONSET OF MITOTIC INHIBITION

The normal time required for the completion of mitosis by the corneal cells is about 70 minutes. If the inhibition of mitosis by HN2 were immediate all normal mitoses would be expected to disappear within this period after the administration of the agent. Allowing a short time for penetration and reaction of the agent, this is approximately the case when concentrations of HN2 hydrochloride are used that are near the necrotizing or vesicant level. With much lower concentrations, near the threshold of mitosis inhibition, the number of mitoses in the cornea is found to decline very much more slowly. Thus for example, with a concentration of .0037%, the number of mitoses reaches a minimum only after 18 hours (Fig. 1).

This slow decline in the number of mitoses might be due either to a slow development of the inhibition following the initial reaction of HN2 with the tissue, or to a slowing down of the progress through the mitotic cycle of those cells that were in mitosis at the time of action of HN2. In order to test this possibility, we have made use of the effect of ether anesthesia previously reported. It had been shown that a brief anesthesia of 10 minutes duration is followed by a period of over an hour during which no new mitoses appear in the cornea. During this period of temporary inhibition, those cells that are in mitosis at the time of anesthesia progress apparently quite normally through their mitotic cycle as is shown by the fact that cells in prophase, in metaphase, etc. disappear in orderly fashion from the picture.

The following experiment was, therefore, performed. One drop of 0.0037% HN2 hydrochloride was instilled into one eye each of a number of rats. As shown in Figure 1, this dose leads to a slow decline in the number of mitoses in the cornea. At various times during this slow decline, the animals were anesthetized for 10 minutes with ether. One hour later they were killed by decapitation and the eyes removed

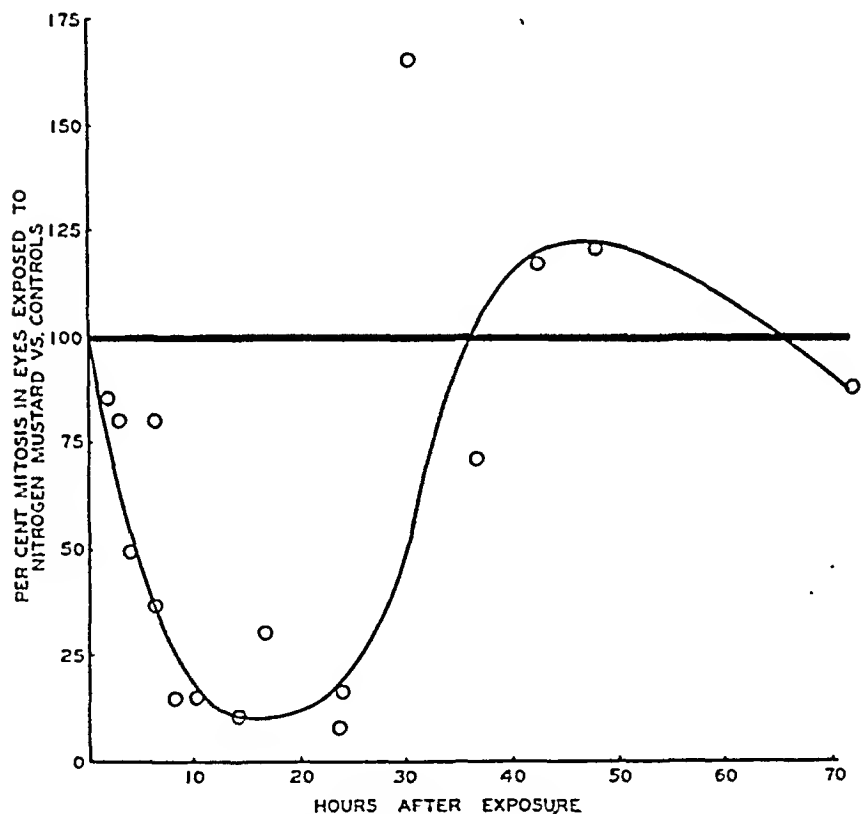


FIG. 1. INHIBITION OF MITOSIS FOLLOWING INSTILLATION OF ONE DROP OF .0037% NITROGEN MUSTARD SOLUTION

for mitosis counts. It was found that the disappearance of mitoses following anesthesia was just as complete in the treated as untreated eye. There is, therefore, no reason to suppose that HN2 in this concentration decreases the rate of progress of cells through mitosis once mitosis has begun. It is concluded, therefore, that the slow decline in the number of corneal mitoses following the administration of very low concentrations of HN2 is due to a slow development of

the inhibition and that this inhibition reached maximum long after the reaction of HN2 with the tissue has run its course.

The implications of this finding in respect to the mechanism of mitosis inhibition require further exploration. One possible explanation may, however, be suggested at this time, namely that HN2 may not directly attack the mitotic mechanism itself but may inhibit the production in the tissue of some substance required for the initiation of mitosis.

With much higher doses of HN2 some slowing down of the mitotic cycle was actually observed. If 0.25% HN2 hydrochloride is instilled into one eye of a rat, the animal then exposed to 10 minutes light ether anesthesia, and the animal sacrificed one hour later, the control eye shows a negligible number of mitoses, while the eye that had been exposed to HN2 still shows an appreciable number. In fact, with this dose of HN2 the last stragglers in the array of mitosing cells have not completed their mitotic cycle even at the end of 3 hours, as is to be seen in Table I. The slowing down of the mitotic cycle with such very large doses of HN2 appeared to affect all phases of the mitosis about equally.

RECOVERY FROM INHIBITION

Spontaneous recovery from the inhibition of mitosis occurs in the corneal epithelium after a lapse of time which varies with the dose of HN2. In Figure 1 it is seen that this recovery can be associated with a transitory rise of the mitosis rate above the normal level. A similar transitory excess of mitoses is seen after recovery from inhibition by ether and some other agents. The larger dose of HN2, the longer the period of inhibition and the slower the recovery. When concentrations as high as 0.03% are used mitotic activity is suppressed for 7-10 days.

CELLULAR GROWTH IN THE ABSENCE OF MITOSES

If a drop of 0.03% HN2 hydrochloride is instilled into rats' eyes every five days, mitoses can be suppressed in the cornea for as long as a month. Throughout the first 2-3 weeks of such an experiment the rats' corneas appear grossly quite normal. By the fourth week, however, some clouding of the cornea and congestion of the conjunctiva

develop along with a moderate mucopurulent discharge. Some of the eyes in this experiment were removed for histological study 10 days after the onset of the experiment. Flat preparations of these corneas, Fig. 2, revealed that the horizontal dimensions of the basal cells and of their nuclei had increased to about double the normal, the area covered by each cell being about four times that of the normal controls; thus the number of basal cells in these eyes was about one

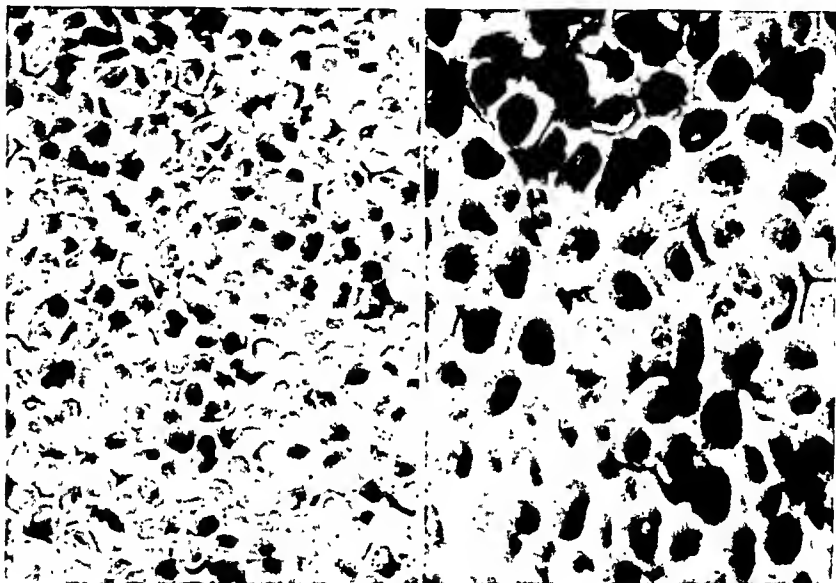


FIG. 2

- a) Normal control showing several mitoses.
- b) Enlargement of corneal cells after 10 days inhibition of mitosis by nitrogen mustard. This microphotograph was taken under the same magnification as Fig. 2a.

quarter that in the normal, and, since the superficial layers of cells had largely desquamated off, the number of epithelial cells was reduced to less than a quarter of those normally present.

Sagittal sections through some of these specimens revealed that the height of the basal cells was about one half that of the normal. With four times the area and half the height the volume of these cells was about twice that of those in normal controls. Since the average inter-mitotic period in the basal cells of the rat's corneal epithelium is

normally about 7-10 days, it is evident that the growth of cell volume during this 10 day period of mitotic inhibition was not far short of that required for normal multiplication. Those eyes had received two doses of HN2, one on the first and one on the sixth day of the experiment.

If no further doses of HN2 are administered, recovery from the mitotic inhibition occurs in 3-6 days more and, by the 17th day, eyes removed from histological study showed basal cells of normal size and number though the full normal thickness of the five layers of epithelial cells had not yet been reached.

HEALING OF WOUNDS IN THE CORNEAL EPITHELIUM

Studies previously published from this laboratory (3) have shown that the healing of small pin prick wounds or cuts in the corneal epithelium takes place by migration of the adjacent cells into the denuded area without requiring any cellular multiplication during the actual period of wound closure. Indeed, mitotic activity is actually inhibited for several hours in the corneal epithelium surrounding a pin prick injury and can be suppressed over the entire cornea during the whole period of primary wound closure if a sufficient number of pin pricks are distributed over the surface. It was of interest to see whether this migratory activity was impaired by exposure to HN2.

Experiments were performed at low and at high doses and at various times after exposure to HN2. In all these experiments the rate of healing was found to be normal. It is of particular interest that the healing of pin prick wounds was normal even with doses of HN2 sufficient to produce marked loosening of the corneal epithelium. Fig. 3 shows a partially healed wound under such conditions. The corneal epithelium is so much loosened from the stroma that on fixation it has become detached and slightly folded in the neighborhood of the wound.

Even larger areas of corneal denudation are covered at a normal rate in spite of the absence of mitoses. This is perhaps not as paradoxical as it would at first appear for the corneal epithelial cells can in such instances become greatly flattened, each cell thus covering a much larger area than it normally would. Moreover, coverage of the defect can occur not only by migration into the denuded area of the remaining corneal epithelial cells. Conjunctival epithelium can

also participate in the covering of the defect. Indeed, with larger doses of mustard or nitrogen mustard large portions of the loosened corneal epithelium commonly slough off and the denuded area is often first covered by a migration of conjunctival epithelium into the defect. In pigmented animals whose limbal epithelial cells contain melanin pigment the movement of this line of pigment can often be readily seen. Fig. 4 shows a section through such a pigment line with epithelium of corneal type on one side of the pigmented cells, of con-

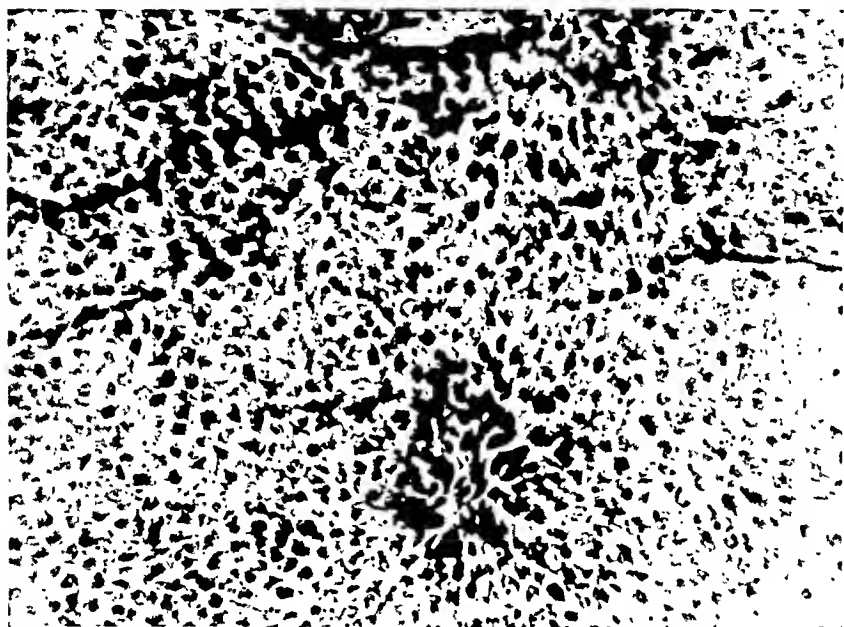


FIG. 3. Microphotograph of a healing pin prick in the corneal epithelium after treatment with nitrogen mustard. The folds in the corneal epithelium are evidence that loss of cohesion has occurred. Nevertheless, the wound healing is normal.

junctival type on the other side. The participation of conjunctival epithelium in the healing of corneal epithelial defects has been discussed in the preceding paper.

SYSTEMIC POISONING WITH HN2

It was of interest to know whether inhibition of mitosis in the cornea could occur if the poison was administered systemically and to compare the mitotic rates in other organs after systemic poisoning with those in the cornea.

Eight rats were used for the following experiment: two received a subcutaneous injection of 1.9 mg. per kilo of HN2 hydrochloride; two received the same dose of HN2 hydrochloride followed 24 hours later by 2 mg. per kilo of colchicine; two received colchicine and no HN2 hydrochloride; two were untreated controls. The animals were sacrificed 8 hours after the colchicine injections, 32 hours after the HN2 hydrochloride injections. Mitosis counts were made on cornea, bone marrow, and small intestine. The results in Table III show that HN2 hydrochloride injected intramuscularly produces a marked

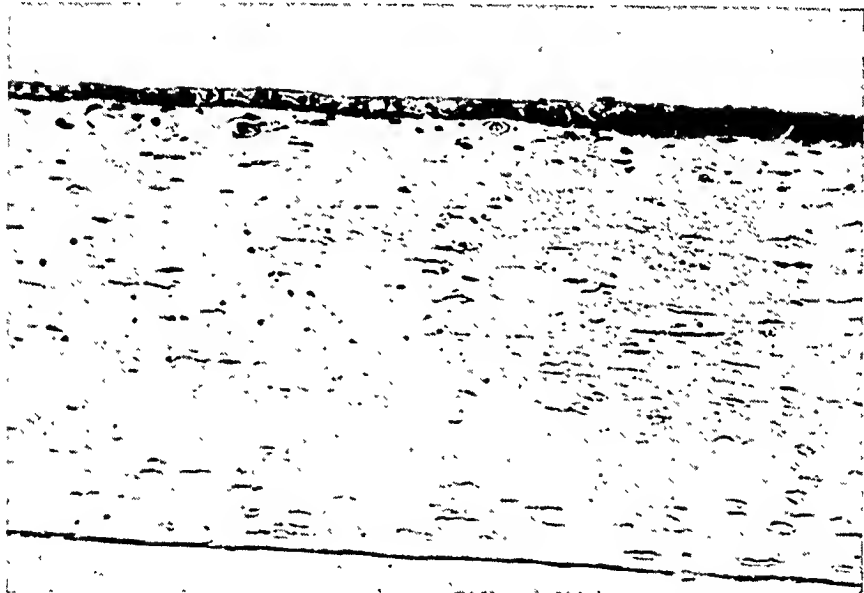


FIG. 4. OVERGROWTH OF CONJUNCTIVAL TYPE OF EPITHELIUM ON THE CORNEA AFTER EXPOSURE TO NITROGEN MUSTARD

inhibition of mitotic activity in cornea, bone marrow, intestine. With dosage used, the effect is most marked on the bone marrow, least marked on the cornea. This may mean that the cornea is more resistant to the effects of HN2 hydrochloride than is the bone marrow, but there may be some question as to whether the same concentration of the agent reaches both tissues after intramuscular injection.

This experiment indicates that inhibition of mitosis in the bone marrow and intestine constitutes a part at least of the effects of HN2

hydrochloride leading to delayed death. With the dosage used, it certainly does not account for the whole effect for, as has been shown by others, this dose leads after a somewhat longer interval to an extremely widespread depletion of bone marrow cells. If we assume that roughly half of the cells in the normal rat's bone marrow are cells which have completed the mitotic phases of their development and are merely maturing before subsequent passage into the blood stream, we cannot account for a disappearance of much more than one-half of all bone marrow cells solely by inhibition of mitosis. Other workers,

TABLE III
Inhibition of Mitosis by HN2 Hydrochlorid Injected Intramuscularly

	NORMAL CONTROL	HN2	COLCHI- CINE	HN2 AND COLCHICINE
<i>Cornea</i>				
Average number of mitoses per section (6 sections per cornea)	1.4	1.1	62.0	16.5
<i>Intestine</i>				
Average number of mitoses per crypts (12 crypts in cross section counted)	0.65	0.2	4.15	0.55
<i>Bone Marrow</i>				
% cell in mitosis	2%	too few to count	40%	too few to count

however, have found that 90% or more of the bone marrow cells disappear following such a dose as was used in this experiment and these lost cells cannot be accounted for by the appearance of immature cells in the blood stream. Moreover, such aplastic bone marrows are loaded with the debris of dead disintegrating cells. It must be concluded, therefore, that a dose of HN2 hydrochloride which leads to extreme aplasia of the bone marrow involves injury to resting cells in the bone marrow as well as inhibition of mitosis.

SUMMARY

Mustard and the nitrogen mustards are powerful inhibitors of mitosis in the corneal epithelium. The inhibition of mitosis can be produced by doses of these agents far smaller than those required to

produce clinically recognizable signs and symptoms of damage. With threshold doses the inhibition of mitosis comes on slowly, reaching a maximum many hours after the primary reaction of the toxic agent with the tissue has been completed. Recovery from the inhibition occurs spontaneously. The duration of the inhibition increases with the dosage applied and can be made to last for several weeks by repeated instillations of the poison. During a prolonged inhibition of mitosis the basal cells of the corneal epithelium increase in size. Wounds in the corneal epithelium heal at a normal rate even in the absence of mitotic activity. Cells that are in mitosis at the time of exposure to the toxic agents complete their division normally and at normal speed unless the dose applied is very large, under which circumstances some slowing down of the whole mitotic cycle occurs. Systemic administration of the poisons in approximately MLD 50 dosage causes an inhibition of mitosis in the corneal epithelium and also in the bone marrow and intestinal mucosa. Inhibition of mitosis represents the lowest threshold effect so far recognized in the reaction of tissues to these poisons.

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V. NUCLEAR FRAGMENTATION PRODUCED BY MUSTARD AND NITROGEN MUSTARDS IN THE CORNEAL EPITHELIUM*

JONAS S. FRIEDENWALD AND WILHELM BUSCHKE

In the preceding paper mention was made of a peculiar mode of death of some cells in the corneal epithelium after exposure to mustard or nitrogen mustard. The threshold dose at which this phenomenon first makes its appearance is 10–20 times that for mitosis inhibition. As will appear from the more detailed description below this is a form of karyorrhexis, a mode of cellular death that has been described in other tissues in a variety of conditions. We have preferred in this paper to call the phenomenon that we were observing by the non-committal descriptive term of nuclear fragmentation, avoiding thereby any implication that the mode of development and nature of this particular pathological process are similar to those of other instances of karyorrhexis that we have not investigated. Similarly disintegrating cells have been noted by others in the bone marrow and the other organs in systemic mustard poisoning. The present study was undertaken in the hope of throwing light on this peculiar pathological process and of determining whether and in what way the phenomenon was related to mitotic activity.

I. DISTRIBUTION AND FREQUENCY OF NUCLEAR FRAGMENTATION

Isolated cells showing nuclear fragmentation are occasionally seen in the corneal epithelium of normal rats. Very extensive and numerous cell changes of this type are seen after exposure to ultraviolet light. In colchicine poisoning, in which mitosis is arrested in metaphase, dispersal of the chromatin can occur if the exposure to colchicine is prolonged. The final picture of such nuclear fragmentation under colchicine resembles the final picture after mustard and ultraviolet exposures, but, as will be noted below, the cytological steps by which the nuclear fragmentation is reached are not the same.

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

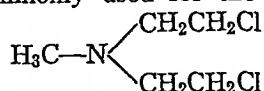
After instillation into rats' eyes of one drop of .03% or more of HN2¹ hydrochloride, cells in nuclear fragmentation are frequently seen. Owing to the frequent extracellular scattering of the nuclear fragments only very rough estimates of the number of affected cells can be given. They are found only in the basal layers of the epithelium² and usually about .04–1.0% of the basal cells show this change, although under special conditions, as will be noted below, the number of affected cells can be considerably increased. Even if the dosage of HN2 is increased to 1% or more, sufficient to produce complete destruction of the eye, the percent of cells showing this type of death is not appreciably increased though cells undergoing pycnosis appear in increasing numbers. Moreover the incidence of nuclear fragmentation following the same dose of HN2 varies markedly from animal to animal. If the eyes are enucleated, dipped for 10 minutes in solutions of .00125 to 0.03% HN2 hydrochloride in M/1000 HCl, and then incubated in moist chambers, nuclear fragmentation appears with much greater regularity and in a much larger number of cells.

These experiments made it clear that in the corneal epithelium only cells in a particular physiological state are susceptible to this kind of injury. It may be pointed out that in the corneal epithelium only cells in the basal layers undergo mitosis and that the number of mitosing cells normally found is of the same order of magnitude (0.4%) as the number which can be thrown into nuclear fragmentation by exposure to HN2.

II. CYTOLOGICAL STUDIES

By using the supravital technique with which nuclear fragmentation is regularly produced in abundance it is possible to obtain material in which the cytological development of the process may be studied. The first visible alteration is seen one to two hours after exposure, at which time scattered cells are found in which the nuclear chromatin shows a fine granular appearance. As time goes on the granules

¹ HN2 is the symbol commonly used for the nitrogen mustard



² With very prolonged supravital incubation similar changes also occur in other cell layers.

become larger and fewer in number, and tend to accumulate on the nuclear membrane leaving a clear homogenous non-basophilic material in the nuclear space between them. At this stage many of the affected nuclei appear somewhat larger and rounder than normal (Fig. 1). About four hours after exposure the chromatin in some of the affected cells has all become aggregated into a few lumps and in some of the affected cells the nuclear membrane has disappeared. At this stage the appearance of the cell is much like that of normal mitosis in

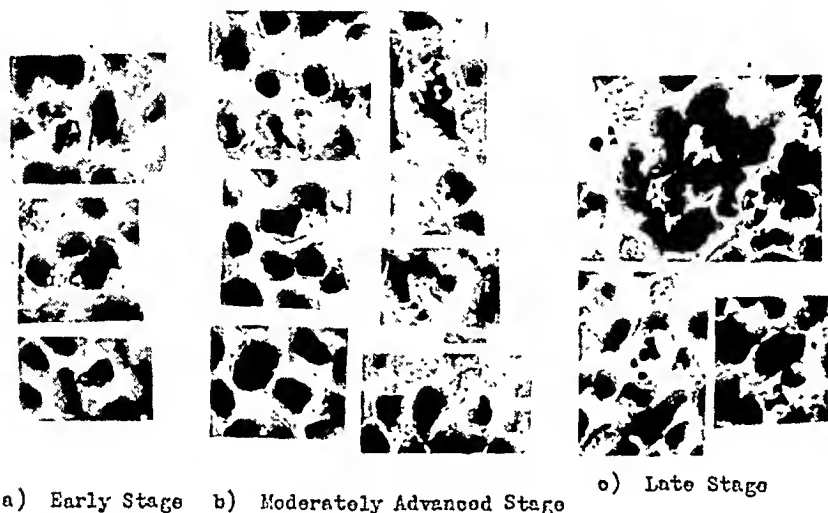


FIG. 1. NUCLEAR FRAGMENTATION IN THE RAT'S CORNEAL EPITHELIUM FOLLOWING EXPOSURE TO NITROGEN MUSTARD

metaphase with the chromatin lying in a zone of clear material in the center of the cell, only instead of chromatin being arranged in an orderly pattern of chromosomes, it is aggregated in irregular amorphous masses and no spindle is formed. The aggregated chromatin is Feulgen positive and the amount of Feulgen positive material in these cells is greater than in normal resting cells. Not all exposed cells reach this stage simultaneously, even at 4-6 hours after exposure one sees many cells in the prophase-like stage of chromatin aggregation within a still preserved nuclear membrane.

After the disappearance of the nuclear membrane the picture departs more and more from that of a normal mitosis. No spindle is formed. We have not been able to demonstrate regularly centrioles or astral

bodies in normal mitoses in the corneal epithelium, and can therefore attach no significance to the fact that these are also not seen in the process of nuclear fragmentation. With time the clear zone of nuclear material in the center of the cells disappears and the fragments of chromatin become dispersed throughout the cell body. Some cells in this stage appear swollen, others shrunken. Eventually the cell wall is ruptured and the cell debris discharged.

From the morphological point of view the process which leads to nuclear fragmentation can be described as a pathological and incompleated mitosis. Previous studies have shown that in the corneal epithelium the time required for a normally mitosing cell to pass through prophase and enter metaphase is one half hour or less (1). The pathological mitosis after exposure to HN2 requires upwards of two hours to proceed through comparable phases. There are evidently close analogies between the phenomenon that we are studying here and the prolongation of mitosis in invertebrate eggs found by Cannan (2) and coworkers after exposure to HN2.

III. EXPERIMENTS ON THE INHIBITION OR ARREST OF NUCLEAR FRAGMENTATION

Nuclear fragmentation following exposure to HN2 is prevented or delayed by incubation of the tissue at temperatures lower than the normal body temperature (Fig. 2). Quantitative studies on the nuclear fragmentation produced by exposure to ultraviolet light, reported elsewhere (3), show that the temperature coefficient for the development of this phenomenon is very large ($Q_{10} = 3.5$). A similar temperature coefficient was found for nuclear fragmentation produced by HN2.

Nuclear fragmentation following exposure to HN2 is prevented or delayed by anoxia. Nuclear fragmentation following exposure to ultraviolet light is likewise suppressed by anoxia.

In a series of experiments freshly enucleated rats eyes were dipped for 10 minutes in HN2 hydrochloride, freshly dissolved in isotonic phosphate buffer (pH 7.4), and then briefly washed in normal saline solution. Following this the eyes were placed in an incubator at 37°C, some in a moist chamber, some in aerated phosphate buffer, and some returned to freshly prepared HN2 solution which was aerated. After

5 hours of incubation they were fixed, and stained. Those incubated in the moist chamber showed the usual number of fragmented nuclei. Those in the phosphate buffer with or without the added HN2 showed no nuclear fragmentation. These experiments suggest that some factors necessary for the fragmentation process are removed in the bathing fluid.

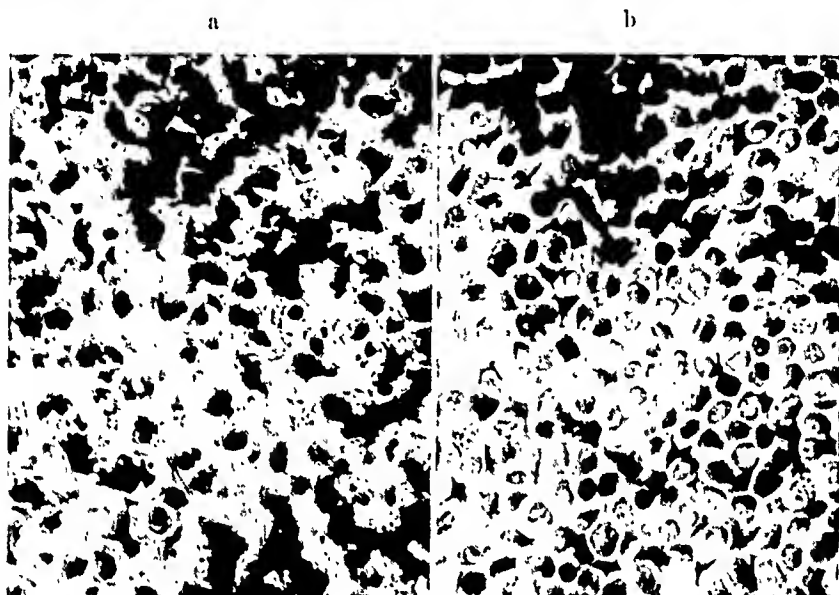


FIG. 2. CORNEAL EPITHELIUM AFTER EXPOSURE TO NITROGEN MUSTARD

a) Incubated at 37°C.

b) Incubated at 10°C.

It is concluded that the process of nuclear fragmentation involves certain aspects of the normal metabolic processes of the cells,—oxygen, normal incubation temperature, and a substance or substances extractable on washing the tissue. Whether the oxidative reaction concerned in this pathological process consists in the oxidation of some substrates not normally accessible to the respiratory chain of reactions, or whether some normally counterbalancing reductive process is inhibited can not be determined. As will be seen from the studies on corneal metabolism that are presented in later papers of this series, Dr. Herrmann has found no measurable disturbance in the overall metabolism of the tissue after exposure to doses of mustard

which produce only nuclear fragmentation and mitosis inhibition. The metabolic disturbance which is related to this effect is, therefore, a highly specific one involving only a small fraction of the total metabolic activity of the tissue. It may be noted, however, that low temperature, anoxia, and incubation of the tissue in aerated solutions also inhibits mitotic activity. It may be, therefore, that the inhibition of nuclear fragmentation by these agents operates through an inhibition of some factors necessary for normal mitosis, but no such positive conclusion is justified from experiments with these highly unspecific inhibiting procedures.

V. RELATION OF NUCLEAR FRAGMENTATION TO NORMAL MITOSIS

The question of the locus of injury which leads to nuclear fragmentation may be approached from several points of view. One may ask what part of the cell is injured or what cellular process is inhibited. One may ask at what phase of the cycle leading to cell division is the cell susceptible to this special type of injury. Finally, one may ask at what stage in the cycle of cell division does the process begin to deviate from the normal. All three of these questions are closely interlinked and have to be approached piecemeal, and only incomplete answers can be reached at the present stage of the study.

a) Effect of HN2 on cells in mitosis. The question of whether nuclear fragmentation results from an injury by HN2 to cells in the process of mitosis at the time of exposure is readily approachable. A variety of agents has been found:—adrenalin, ether anesthesia trauma, morphine, which prevent the entrance of cells into mitosis but do not interfere with the progress of cells that have already entered mitosis (1, 4). After administration of one of these agents the number of mitoses found in the cornea steadily declines to zero. First prophases disappear, then metaphases, etc., until about an hour after the onset of the inhibition no mitoses remain in the tissue. If the tissue is exposed to HN2 before or simultaneously with one of these mitosis inhibitors and samples are examined at various intervals after exposure, it is possible to determine whether the exposure to HN2 has altered the progress of cells through mitosis. Experiments of this type, similar to those reported in the previous paper, revealed

a slight slowing in the progress of cells through the mitotic cycle, but all normal mitoses disappear before any cells in the earliest recognizable state of nuclear fragmentation become visible. Those cells that are in mitosis at the time of exposure complete the cycle in an essentially normal though slightly delayed manner.

The reverse experiment can also be performed. The rat's cornea can be depleted of mitoses by a previous administration of an inhibiting agent, e.g., adrenalin, kept free of normal mitoses for the whole duration of the experiment, and HN2 administered. In such experiments, nuclear fragmentation develops following HN2 more regularly and more abundantly than when HN2 is given to control rats. It must be concluded that nuclear fragmentation does not develop from injury by HN2 to cells already in mitosis at the time of administration. The locus of the injury which leads to nuclear fragmentation, and the locus at which the pathological process diverges from the normal, is antecedent to the histological visible stages of mitosis.

b) Pre-Mitosis. The nature of this pre-mitotic susceptible state can be defined more specifically as follows: Cells which once have reached the histologically recognizable states of mitosis are apparently irreversibly committed to the mitotic process. We may inquire whether the locus of HN2 injury which leads to nuclear fragmentation lies before or after the irreversible entry of the cell into the mitotic process. Evidence on this point can be obtained from an analysis of the inhibition of mitosis produced by adrenalin and other similarly acting agents. When adrenalin is injected intramuscularly into a rat, there results a period during which no new mitoses appear in the corneal epithelium; depending on the dosage the duration of this period varies. By using a suspension of adrenalin in peanut oil the inhibition can be made to last up to 10-12 hours. With the waning of the adrenalin effect mitoses reappear and during the first hours of this recovery period the number of mitoses commonly overshoots the normal level. The overshooting, however, is small and brief and, in our experience, never is sufficient to make good the previous deficit, nor does the overshooting increase with the prolongation of the inhibition. It follows that the locus of inhibitory action of adrenalin on the mitotic cycle is not later than the point of irreversible entry into mitotic activity,

for, if the inhibition acted only at some later point in the cycle, cells would accumulate at the normal rate in the preinhibited state and would escape from this state in the recovery phase, with a resulting excess overshooting of mitosis numbers that should compensate for the deficit accumulated during the period of inhibition.

Since HN2 produces nuclear fragmentation even when an adrenalin inhibition of entrance into normal mitosis exists at the time of HN2 administration and throughout the whole period of development of the nuclear fragmentation, we can conclude that the locus of action of HN2 is antecedent to the irreversible entrance of cells into mitosis. This antecedent state cannot be the normal resting state since only a very few cells in the cornea are at any given moment susceptible to this form of injury. It may be suggested, therefore, that there exists a reversible pre-mitotic state antecedent to the irreversible mitotic state, and that in the corneal epithelium this pre-mitotic state is the source of the cells which after exposure to HN2 undergo nuclear fragmentation.

In order to study this relation quantitatively, experiments were performed in which one drop of 0.25% HN2 hydrochloride was instilled into one each of a series of rats at various times before or after the intramuscular injection of 0.2 mg. of adrenalin in peanut oil. The eyes were removed for histological study 6 hours after the administration of HN2. The animals in these experiments were kept each in a separate cage in the quietest part of the laboratory and were not handled or disturbed for at least 12 hours before the beginning of the experiment. This was done in order to reduce to a minimum the excitement of the rats, and to avoid adrenalemia not caused by the injections. The control rats in this series which received no adrenalin injection, and also those that received the adrenalin simultaneously with, or later than, the HN2, showed minimal numbers of cells with nuclear fragmentation. When HN2 was given 1 to 4 hours after the adrenalin injection relatively great numbers of cells in nuclear fragmentation resulted. Adrenalin alone never causes nuclear fragmentation. Similar results can also be obtained if the inhibition of mitosis is produced by a hypodermic injection of morphine. Under morphine hypnosis the body temperature of rats is markedly lowered. Since decreased temperature had already been shown to delay greatly

the onset of nuclear fragmentation, the animals in this experiment were kept warm in the incubator.

These experiments show that when the normal entry of cells into irreversible mitosis is blocked then some cells accumulate in the pre-mitotic state. This is not in contradiction with the previous conclusion that the susceptible pre-mitotic state is reversible. If cells in the pre-mitotic state normally can escape from this condition either by entering mitosis or by returning to the resting state, then the closing of one of the exits will, other things being equal, lead to an accumulation of cells in the pre-mitotic state until the rate of escape through the remaining exit is equal to the rate of entrance. This is, in fact, in accord with the phenomenon of overshooting regularly seen in the recovery phase after adrenalin inhibition. This conclusion is not unequivocal since, obviously, adrenalin and morphine might increase the number of cells in the susceptible state through some action wholly unrelated to their effects on the mitotic process.

Since in the control animals with minimal adrenalemia the number of fragmented nuclei produced by HN2 is very small, we can conclude that there are few cells in the susceptible state under these conditions, and hence that the normal duration of the susceptible state in the corneal epithelium is quite short in comparison to the whole inter-mitotic period. Not all procedures which lead to an inhibition of mitosis lead to an increased susceptibility to nuclear fragmentation. For instance, if eyes are enucleated and placed in a moist chamber in the incubator, mitoses appear in decreasing numbers during the second hour of incubation, and by the end of three hours and for several hours thereafter no mitoses are visible in the tissue. If, at the end of three hours incubation the eyes are exposed to HN2 and then re-incubated, nuclear fragmentation does not develop. Evidently some factors necessary for the mitotic process and also factors necessary for the fragmentation process are exhausted by this procedure.

Effect of Hypotonicity and Low pH

If freshly enucleated rats eyes are dipped for 10 minutes in a freshly prepared 0.25% solution of HN2 in M/6 phosphate buffer, and then incubated in a moist chamber for 6 hours, only a moderate number of epithelial cells develop nuclear fragmentation, the number being

comparable to that produced in vivo. On the other hand, if the enucleated eyes are dipped in HN2 hydrochloride solution dissolved in M/1000 HCl and then incubated, the number of cells developing nuclear fragmentation is very much greater. A variety of experiments were performed in attempting to elucidate this peculiar finding. If the HN2 is dissolved in 0.9% NaCl containing M/1000 HCl, fewer nuclear fragmentations are produced than in the hypotonic M/1000 HCl solution without NaCl, but more than are produced by exposure to HN2 in the phosphate buffer. If the eyes are dipped into distilled water or M/1000 HCl solution before being placed for 10 minutes in a solution of HN2 in the phosphate buffer, there is a moderate increase in the number of nuclear fragmentations produced. Apparently both hypotonicity and low pH before or during the exposure favor the development of the lesion.

The interpretation of these findings is complicated by the fact that HN2 is transformed more slowly into its reactive derivative, the cyclic ethylene imine, at low pH and in the presence of high concentrations of chloride or phosphate ions, but, since the intracellular ionic changes are probably small compared with those in the bathing fluid, it seems likely that hypotonicity and low pH produce their effect by increasing the number of susceptible cells rather than by radically altering the reactivity of the toxic agent. In this connection it is to be remembered that similar brief exposures to abnormal salt concentrations and acidities have been used by many investigators to precipitate parthenogenesis in various ova.

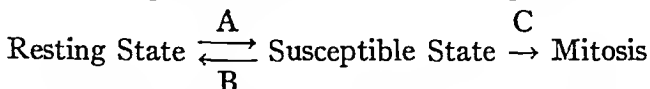
V. LOCUS OF MITOSIS INHIBITION BY HN2

As previously reported, the administration of very small doses of HN2 to rats' eyes results in a slowly declining mitotic activity without the production of nuclear fragmentation. With appropriate dosage (one drop of .004-.008% HN2 hydrochloride) mitoses disappear completely from the corneal epithelium in 18 hours, reappear in about 36 hours, and overshoot the normal numbers about 48 hours following exposure. In a series of experiments a second dose of more concentrated HN2 hydrochloride solution was administered either at the period of mitotic inhibition or at the period of mitosis excess. For controls the opposite eyes of the same animals were used and these

received either only the first small dose of HN2 or only the second large dose of HN2. Six hours after administration of the second dose the eyes were enucleated. It was found that the first small dose caused no nuclear fragmentation and that it resulted neither in an appreciable increase nor in an appreciable decrease of the number of fragmented cells following the second dose. It follows: (1) that the locus of mitosis inhibition by HN2 cannot be antecedent in the mitotic process to the locus of injury producing nuclear fragmentation; and (2) that the locus of mitosis inhibition by HN2 must be different from that by adrenalin and morphine. Consequently the mitosis inhibition must be either at the same pre-mitotic stage as that in which larger doses lead to nuclear fragmentation, or it must be at some stage in the irreversible portion of the mitotic cycle, i.e. at a later stage than the locus of adrenalin inhibition. The former of these two alternatives would seem a priori more likely, and the latter can be excluded by direct experimental test, for if the mitosis inhibition of HN2 occurs at a later stage in the mitotic cycle than the inhibition of adrenalin, then the overshooting of mitosis numbers during the recovery from HN2 inhibition should be relatively refractory to adrenalin inhibition. Experiments revealed that mitotic activity during the recovery phase following HN2 inhibition is just as susceptible to adrenalin inhibition as is mitotic activity normally. The conclusion that the locus of mitotic inhibition by HN2 is the pre-mitotic state is in agreement with the findings of Cannan on invertebrate eggs.

DISCUSSION

The course of events in the normal cycle which is indicated by this analysis may be represented schematically as follows provisionally identifying the susceptible state with that of pre-mitosis:—



Adrenalin, morphine, cause an inhibition at (C) leading to an accumulation of cells in the susceptible state. Cells in this condition when exposed to larger doses of HN2 are damaged in such a way that after some hours they undergo a pathological process leading to nuclear fragmentation. Even with smaller doses of HN2 progress

through the pre-mitotic state toward normal mitosis is inhibited. A simple hypothesis that is compatible with all the data so far obtained, is that in the larger doses HN2 inhibits the return of cells from the pre-mitotic state to the resting state. Such an hypothesis presupposes that the pre-mitotic state is essentially unstable, and that cells frozen in this state, unable either to return to the resting state or to escape into normal mitosis proceed through such portions of mitotic activity as they are still capable of.

The notion of an intrinsic instability of the pre-mitotic state receives some support by analogy from the experiments of Loeb (5) on parthenogenesis which have been confirmed and extended by many other investigators. These experiments show that cells exposed to agents which stimulate parthenogenesis tend to undergo cytolysis. If they are protected from cytolysis by what Loeb called "compensating agents" parthenogenesis results. This analogy must not be pressed too far for the cytolysis which has been observed in these experiments is a very different process from that of nuclear fragmentation. Special susceptibility of cells in pre-mitosis to X-ray injury has been suggested by Strangeways and Oakley (6), and our own experiments do not exclude the possibility that cells in this state are directly damaged by the HN2, not merely frozen in an unstable state.

If susceptibility to nuclear fragmentation on exposure to HN2 can be taken as a sign that a cell is in the pre-mitotic state, then the number of cells normally present in this state in the corneal epithelium is small when the animals are in basal condition (no adrenalemia), roughly one tenth the number normally present in the histologically recognizable state of mitosis. Assuming that at least as many cells enter this state per hour as enter mitosis it can be concluded that the normal duration of the pre-mitotic state is about one tenth the duration of normal mitosis, i.e., no more than 5-10 minutes. Again judging from the maximum number of cells which show fragmentation when HN2 is administered after various inhibitors of mitosis one can conclude that cells may stay in the premitotic state for an hour or two under these conditions.

The analysis of our experiments furnishes an explanation for the fact that rapidly growing tissues are susceptible to injury by mustard. It has previously been supposed that cells in mitosis are particularly

susceptible. Our studies on the influence of various agents on cells already in mitosis show them to be remarkably resistant. The pre-mitotic state, on the other hand, is highly vulnerable to some agents.

The visceral lesions of systemic mustard poisoning appear to concern primarily those tissues in which the mitosis rate is high. It would seem likely that many of these lesions could be interpreted as the results of pathological mitosis and nuclear fragmentation. Certainly the changes in the bone marrow suggest such an interpretation. To some degree, therefore, tissue susceptibility to mustard may be related to the frequency of cells in pre-mitosis in different tissues.

In the foregoing discussion we have provisionally identified the susceptible state in the corneal epithelium with the state of premitosis. The reasons for suggesting this identity are: (1) Nuclear fragmentation due to mustard occurs in the corneal epithelium almost exclusively in the normally mitosing layer. (2) The morphological steps through which nuclear fragmentation develops bear certain analogies to the prophase of normal mitoses and are associated with an increase in the Feulgen positive material in the chromatin. (3) Various non-specific processes which inhibit the mitotic process,—low temperature, anoxia, washing the tissue in inert solutions,—also prevent the development of nuclear fragmentation. (4) Certain drugs which, without causing major damage to the tissue, inhibit the visible onset of mitosis and facilitate the accumulation of cells in the susceptible state. (5) Some other tissues, (e.g. bone marrow) with a high mitotic rate, are particularly susceptible to injury by mustard and show evidence of a similar mode of cellular death.

While the arguments listed above are cogent, though not conclusive, in respect to the phenomena we have studied in the corneal epithelium; it must be definitely stated that this argument cannot be generally extended to other tissues. Certain exceptions can already be noted.

The endothelium of the cornea has a low rate of mitosis, in fact we have never seen a mitosis in this tissue though mitotic divisions may occur during regeneration following injuries. Nevertheless, after exposure to adequate doses of mustard or HN2, the cells of the corneal endothelium in the exposed area uniformly show nuclear fragmentation. The same uniform susceptibility to this type of death is shown by wandering cells, both monocytes and polymorphonuclear

leucocytes, which are normally present in the corneal stroma of animals. The similarity of the process in the corneal endothelium and in the wandering cells in the corneal stroma to that which we have been studying in the corneal epithelium is further shown by the fact that anaerobic incubation suppresses the lesion in all three cell types. Thus the state of susceptibility to nuclear fragmentation following exposure to the mustards cannot, in the corneal endothelium at least, be identified with the state of premitosis. To do so would require the wholly unwarranted assumption that these normally not mitosing cells are constantly in a state of premitosis.

Moreover, not all actively mitosing cells are susceptible to this type of injury. By partial hepatectomy it is readily possible to provoke a high rate of mitosis in the remaining liver tissues. We have performed experiments in which, during the period of maximal mitotic activity following partial hepatectomy in a rat, a MLD 50 dose of HN2 was injected intramuscularly. The animals were sacrificed 24 hours later. Their bone marrows showed extensive evidence of nuclear fragmentation but the regenerating liver was free from any such lesion. It follows that the liver cells, even when actively multiplying are not specially susceptible to this type of injury.

Finally it must be pointed out that ultraviolet radiation produces in the corneal epithelium large numbers of cells with nuclear fragmentation, the morphological characteristics of which closely resemble those produced by the mustards, but after exposure to ultraviolet radiation it is the more superficial layer of not normally mitosing cells which show this lesion while the basal cells largely escape.

It follows from all this that while the pathological process which leads to nuclear fragmentation involves some aspects, for instance the elaboration of Feulgen positive material, which are normally a part of the mitotic process, and while, in the corneal epithelium susceptibility to this type of injury by the mustard agents is suggestively connected with a state of premitosis, the identification of the susceptible state with that of premitosis holds neither for some other tissues nor for some other injurious agents.

In the preceding article it was pointed out that nuclear fragmentation occurs in the corneal epithelium with doses of mustard or nitrogen mustard insufficient to produce clinically recognizable signs of damage

in the rat's eye. It should be remembered, however, that in human beings pain, photophobia, lachrymation, and fine punctate stippling of the corneal surface are produced by doses of these toxic agents much smaller than those required to produce gross opacification of the cornea, loosening of the epithelium and purulent conjunctival discharge. These latter signs of a severe necrotizing lesion were the ones on which we had to depend for the recognition of clinically evident damage in the rat's eye. It would seem likely, therefore, that the irritative symptoms of sub-necrotizing doses in human beings are associated with the occurrence of nuclear fragmentation in the epithelial cells. Since more direct evidence of the connection between nuclear fragmentation and irritative symptoms is available in respect to ultraviolet light burns of the eye, this point will not be argued further here.

SUMMARY

1. Nuclear fragmentation is a mode of cellular death induced in the corneal epithelium by threshold doses of mustard and nitrogen mustard. Most of the experiments reported here were performed with HN2, but similar results were obtained with mustard and other nitrogen mustards. When the toxic agent is applied *in vivo*, only cells in the basal layers exhibit this type of reaction and only a small percent of the basal cells are affected, the numbers and location of the affected cells being similar in order of magnitude to those normally found in mitosis. Increase in the dose of the toxic agent does not increase the number of cells showing this mode of death beyond a certain low limit.

2. The cytological changes involved in the development of nuclear fragmentation are remarkably similar to those of prophase and metaphase in normal mitosis. It is suggested that the phenomenon of nuclear fragmentation in these experiments may be a form of pathological mitosis.

3. The development of nuclear fragmentation after exposure to mustard is inhibited by lowered temperature and by anoxia. Both of these experimental conditions likewise inhibit progress through the normal mitotic cycle. Immersion of the tissue in Ringer's fluid or phosphate buffer suppresses the nuclear fragmentation, presumably

by extracting some necessary substances from the tissue. Similar immersion likewise suppresses mitosis in this tissue. Supravital maintenance in a warm moist chamber for three hours exhausts the capacity of the tissue to produce new mitoses. After such supravital maintenance, exposure to HN2 fails to produce nuclear fragmentation.

4. On the basis of experiments reported, it is postulated that the passage of cells from the resting state through mitosis involves two steps: (a) a state of excitation (pre-mitosis) from which they can enter mitosis or return to the resting state; (b) actual mitosis which, once begun, cannot be readily reversed. Experiments with mustard and nitrogen mustard applied to the cornea, under conditions of mitosis inhibition by various other agents, demonstrates that nuclear fragmentation does not result from injury by mustard to cells already in mitosis, but that it is probably the cells in pre-mitosis which, on exposure to mustard, undergo nuclear fragmentation. The pre-mitotic state is not the sole and sufficient condition for susceptibility to nuclear fragmentation from exposure to mustard. There are some tissues, for instance the corneal endothelium, whose cells show a uniform susceptibility to this type of injury in spite of a very low rate of mitosis. There are other tissues, for instance the liver, whose cells show a high resistance to this type of injury in spite of a high rate of mitosis induced by partial hepatectomy. On the other hand, the bone marrow and intestinal mucosa show both a high susceptibility to nuclear fragmentation and a high rate of mitosis.

5. A simple hypothesis that will explain the present findings is that cells in pre-mitosis are, after exposure to mustard, unable either to enter normal mitosis or to return to the resting state, and that after being held in the pre-mitotic state unduly long they proceed into a pathological mitosis which ends in nuclear fragmentation. This hypothesis presupposes that the pre-mitotic state is an unstable one in which the cell cannot long maintain its equilibrium. An alternative hypothesis is that exposure to mustard directly damages cells in the pre-mitotic state and thus induces the pathological process leading to nuclear fragmentation.

6. Brief exposure of the tissue to hypotonic solution, to solutions of low pH, and particularly to HN2 dissolved in hypotonic solutions of low pH, greatly increase the number of cells susceptible to this injury.

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VI. NOTE ON KARYOLYSIS OF THE CORNEAL STROMA CELLS*

JONAS S. FRIEDENWALD

It was noted in the preceding paper that wandering cells normally present in the corneal stroma of rats' and beef eyes undergo nuclear fragmentation upon exposure to mustard and nitrogen mustard, but that the stroma cells, sometimes called corneal corpuscles or keratocytes, do not show this mode of death. With adequate dosage the stroma cell nuclei swell, become pale staining with margination of the chromatin, and eventually burst (Figs. 1-4). Evidence of this type of change can be seen in ordinary histological sections as noted in one of the preceding papers (1). These phenomena are seen only at dosage levels that cause easily recognizable clouding of the cornea and often cause permanent corneal scarring. Similar modes of death of the corneal stroma cells are seen with a wide variety of injurious agents,—arsenicals, alkali burns, freezing, etc.

The phenomenon can be observed in enucleated eyes kept in a moist chamber in the incubator, in which case infiltration with inflammatory cells is avoided and the histological picture rendered more simple, and is most readily observed in flat sections of the tissue or in flat preparations such as were used in the previous paper. For the latter purpose the tissue, after fixation, is soaked for one hour in a saturated aqueous solution of amyl alcohol. After removal from this solution the corneal epithelium and endothelium are found to be markedly loosened and can readily be wiped off, yielding a clean preparation of the corneal stroma which can be stained, cleared, and mounted without sectioning. Most of the experiments reported in this paper were performed on rats. If only the cornea is incubated in a moist chamber neither swelling nor bursting of the nuclei is observed. If corneas, previously exposed to mustard or nitrogen mustard, are incubated not in a moist chamber but in physiologic saline solution, the stroma nuclei swell but do not burst. If normal corneas with uninjured epithelium and

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.



FIG. 1



FIG. 2

FIG. 1. Stroma cells of rat's cornea incubated 7 hours in phosphate buffer (control). The denuded corneal stroma was fixed, stained, and cleared without sectioning.

FIG. 2. Stroma cells of rat's cornea incubated 9 hours in phosphate buffer after exposure to nitrogen mustard. Same magnification as Figure 1.



FIG. 3



FIG. 4

FIG. 3. Same as Fig. 2, incubated for 12 hours showing bursting nuclei.

FIG. 4. Same as Fig. 2, incubated for 16 hours. Only nuclear debris remains.

endothelium are incubated in saline very little swelling of the stroma nuclei takes place, but if normal corneal stroma denuded of endothelium and epithelium is incubated in saline the stroma nuclei swell but do not burst.

These observations lead to the conclusion that extra fluid must enter the corneal stroma in order to enable the stroma nuclei to swell. As long as the epithelial and endothelial barriers remain normal, the normal state of detergescence of the corneal stroma is maintained even supravivally (2). If, however, these barriers are injured then swelling of the nuclei can take place. All of the agents, which in our experience cause swelling of the stroma nuclei, cause a loosening and eventual sloughing of the epithelium and endothelium. However, normal stroma nuclei in the presence of available fluid swell but do not burst. Consequently, after exposure to mustard and other similarly injurious agents the fragility of the nuclear boundary is greater than normal and the nuclei burst. The fact that this bursting of the nuclei occurs in vivo or on incubation of the whole eye, but not on incubation of the isolated cornea, indicates that the ocular tissues or fluids provide some factor, possibly a metabolite, necessary for this pathological process.

It was of interest to discover whether the increased fragility of the stroma nuclei was an immediate or delayed effect of the toxic injury. Experiments were performed in which the corneal endothelium in enucleated eyes was injured mechanically and the eyes then kept for 16 hours in a refrigerator. At the end of this period samples were examined and the stroma nuclei were found swollen but not bursting. Some of these eyes were then exposed to HN2 hydrochlorid and incubated in a moist chamber at 37°C. Samples removed for histologic study at varying intervals showed that the bursting of the swollen stroma cell nuclei occurred about 10 hours after exposure to the HN2, that is at about the same time following exposure as in experiments in which there was no corneal oedema at the time of exposure.

Nuclear fragmentation and karyolysis are not the only modes of cellular death following exposure to mustard. With very large doses of mustard or nitrogen mustard pycnosis of all types of corneal cells is observed, increasing in frequency with increasing dosage.

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VII. THE ADHESION OF EPITHELIUM TO STROMA IN THE CORNEA*

HEINZ HERRMANN AND FAY H. HICKMAN

The advantages of the cornea as a test object for the study of cellular physiology are demonstrated by investigations in many fields. The geometrical simplicity of its structure and the toughness of its connective tissue stroma render it almost uniquely favorable for the study of certain aspects of cellular cohesion. The cohesion of cells and tissues is one of the fundamental factors in the biological organization. However, the phenomena concerned with cellular cohesion have been the subject of only a few experimental studies. Fenn (1) measured the adhesiveness of leucocytes to various solid surfaces, such as glass and carbon. Fenn (2) and several other authors (3, 4) have attempted to relate the magnitude of the adhesive forces to the decrease of the surface tension.

Our experiments deal with the cohesion of the basal layer of the epithelium to the stroma of the cornea. The bulk of the stroma is made up of collagen fibres which give to the stroma its great mechanical strength. The stroma cells lie scattered between the collagen bundles and are entirely absent in the most superficial layers of the stroma. In several species the epithelium is separated from the stroma by a distinct membrane known as Bowman's membrane, but in some species this membrane is absent. In the beef corneas, which were used in our experiments, no morphologically distinct Bowman's membrane is present.

An epithelium supported by and adherent to a fibrous stroma is a very common pattern of histological organization. In most cases the epithelium and the stroma form a morphologically complex aggregate, as for example the tubules in the kidney, the acini in various glands, and the villi in the intestine. In the cornea the separating boundary between stroma and epithelium forms a smooth plane of simple configuration. This simplicity of the boundary and the leath-

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

ery consistency of the stroma make it possible to remove the epithelium quantitatively and measure the adhesive forces under different experimental conditions.

TECHNIQUE

a. *Measurement of Adhesion.* Under certain experimental and pathological conditions the adhesion of the epithelium to the stroma

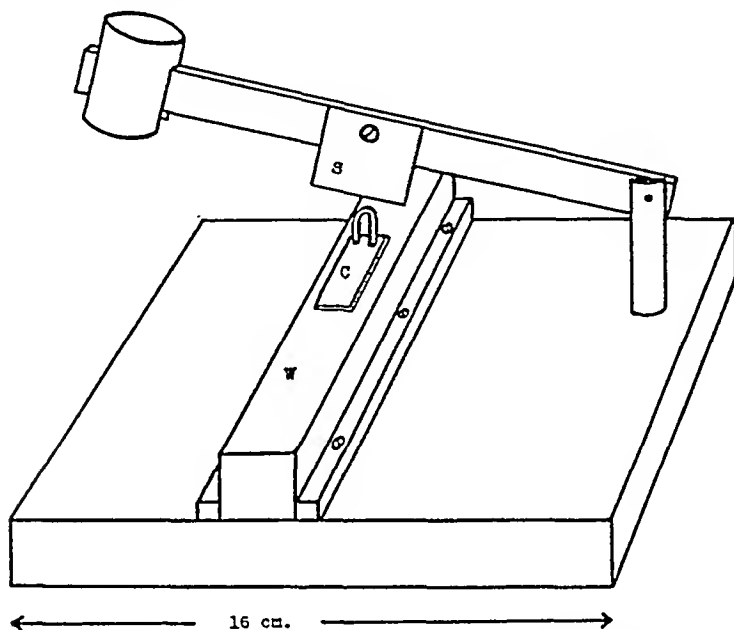


FIG. 1. SCRAPER FOR TESTING THE ADHESION OF THE CORNEAL EPITHELIUM

s. scraper blade

c. corneal strip

w. sliding wood block

is so greatly reduced that the epithelium can be wiped off with great ease, and may, in fact, be displaced by a gentle blink of the lids. In order to place this qualitative observation on a more objective and quantitative basis a simple mechanical scraper was used.

The scraper (Fig. 1) consists of a blade (S) of rustless steel attached to a lever arm. The blade is 0.2 mm. thick and the scraping edge is bevelled at an angle of about 70° . By lowering the lever arm the blade can be brought to rest on a corneal strip (C) secured by a staple

to a smooth wooden block (W). The pressure exerted on the cornea by the blade can be varied by changing the position of a weight on the lever arm to which the scraper is attached. A brass cylinder weighing 99.5 gm. was used as a weight. The force with which the blade rests on the cornea was determined by means of a balance for the unweighted lever and for 5 standard positions of the weight. The pressures used were 40, 60, 95, 110, 165, 200 gm. respectively.

Corneal strips, about 8 mm. in width, were excised along the greatest diameter of beef corneas. The width of the corneal strips were kept as uniform as possible in order to avoid variation in the bearing surface on which the scraper blade rests. The corneal strip which was to be tested was fastened at one end to the wood block by means of an ordinary wire staple, and straightened and flattened out with a glass rod moistened with physiologic salt solution. The scraper blade was then lowered to the corneal surface as near to the staple as possible. The bevelled edge of the blade faces toward the staple; the vertical edge faces the free corneal surface. By thrusting the blade through the epithelium to the surface of the stroma, the epithelium can be incised and an upright epithelial edge established. The blade was then once more lifted above the epithelial surface, the weight on the lever arm adjusted to the desired position, and the blade reinserted into the incision. The wood block with the attached strip of the cornea was then pushed under the scraper (Fig. 1a). Depending upon the weight of the lever and upon the adhesion of the epithelium, the blade will either glide over the edge of the incision in the epithelium and slide over the surface, or the epithelium will be pushed off in front of the blade, which then moves along the surface of the stroma. The speed with which the wood block is pushed under the blade is not critical within wide limits. We completed one stroke in approximately one half second. In removing the epithelium with the scraper the separation generally takes place at the boundary between stroma and epithelium and not between the epithelial layers themselves. The removal of very small areas of epithelium can readily be recognized since the glossy appearance of the epithelium is replaced by the dull stroma surface.

The epithelium which has been removed with the scraper can be collected without difficulty. Any epithelium which remains adherent

to the corneal strip is taken off with a sharp scalpel. By comparing the amount of epithelium removed by the scraper with that subsequently removed by the scalpel, the percent removed by the scraper may be calculated, thus rendering the measurements independent of the total area of the strip that is used.

The amounts of epithelium removed by scalpel or scraper were measured in most experiments by determining the protein nitrogen of the samples by the micro Kjeldahl method. In one series of experiments in which we tested the effect of a great number of substances over a wide range of concentrations, an approximate esti-

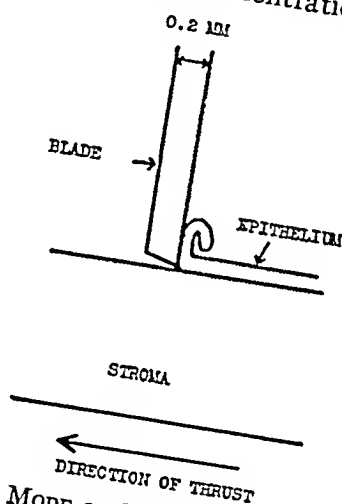


FIG. 1a MODE OF OPERATION OF SCRAPER

mate was made by determining visually whether more or less than one half of the epithelium had been removed from the strip at a given pressure. This procedure was very efficient, and, when used with the practice obtained in several hundred tests, it was sufficiently accurate for the particular purpose.

With a moderate constant weight on the scraper (90, 110 gm.) successive strokes remove approximately the same amount of epithelium until the supply is almost exhausted (Fig. 2). With variable pressure and a fixed number of strokes, the amount of epithelium removed is not a linear function of the pressure. A critical region is found in which a small change in pressure causes a large change in amount removed. We found that with a weight on the scraper of

less than 90 grams very little epithelium is removed in the standard procedure. With a scraping weight of 200 gms. a large part of the epithelium is removed (Fig. 3). When the adhesion of the epithelium to the stroma is decreased, under certain experimental conditions, the critical range of the scraping weight which causes the disruption of the cohesive forces is found at a lower range than in the normal controls. By measuring the per cent of epithelium removed with different scraping weights the range of this critical zone can be determined.

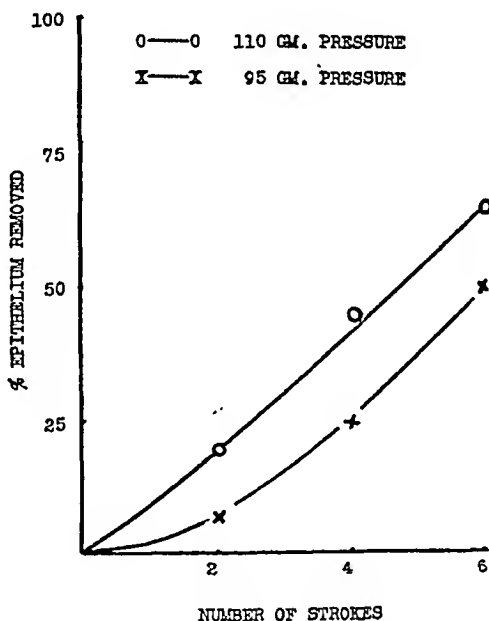


FIG. 2 PERCENT OF EPITHELIUM REMOVED WITH INCREASING NUMBER OF STROKES

b. *Manipulation and maintenance of the cornea.* Beef eyes obtained from the slaughter house arrived at the laboratory not longer than three hours after the death of the animal.¹ Each eye was washed shortly with running lukewarm tapwater. Eyes with opacities, cuts, or other defects on the cornea were discarded.

Some tests were conducted by immersing the excised cornea directly in 25–50 ml. of various solutions.² The controls in these tests were

¹ We are deeply indebted to the management and employees of Schluderberg-Kurdle Co., meat packers who supplied us with the beef eyes and who spared no effort to enable us to obtain the material in the freshest possible state.

² If not indicated otherwise all solutions were adjusted to a pH of 7.2–7.4.

immersed in NaCl solutions of about the same ionic strength. This type of experiment was used with substances available in considerable amounts and with which rapid effects were of interest (e.g. various detergents and ions). Substances available only in smaller quantities, or the effect of which had to be tested after longer time intervals, were injected into the stroma. Routinely the injection was carried out while the cornea was still attached to the eyeball. A 1 ml. tuberculin syringe was used with a No. 26 or 27 needle, which was introduced near the limbus at 4-6 symmetrically placed points of

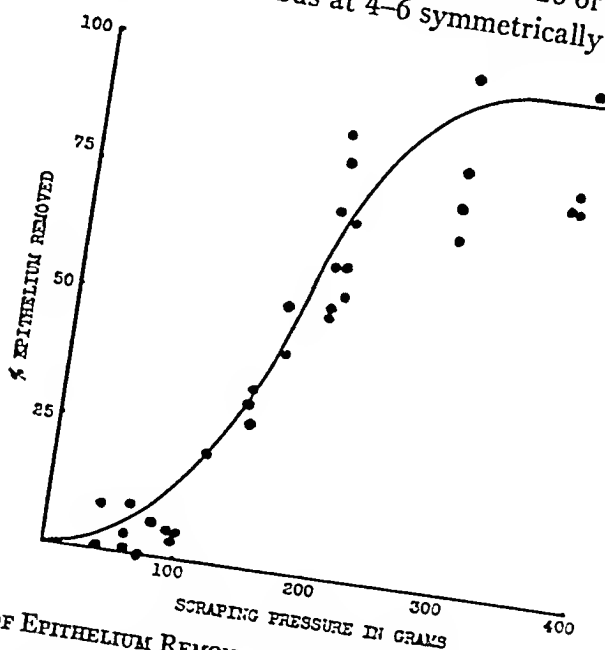


FIG. 3 PERCENT OF EPITHELIUM REMOVED WITH TWO STROKES AND VARIABLE LOAD

the corneal circumference. The point of the needle was forced gently almost to the center of the cornea. The fluid was injected slowly with a minimum necessary pressure and was thus deposited in several more or less confluent areas covering the greater part of the cornea. The total fluid volume injected per cornea was 0.25 ml. About $\frac{1}{2}$ – $\frac{1}{3}$ is lost by drainage, and in assays of the metabolic utilization of injection material the actual amount remaining has to be determined in a sufficient number of controls. We found that 0.45% NaCl solutions, and even distilled water, are well tolerated as solvent for the injected substances. The adhesion of the epithelium is not affected

by injection of 0.25 ml. of such solutions, and the slight bulging and opacity of corneas which follows the injection disappears almost completely on subsequent incubation. A smooth surface is of importance for a satisfactory test with the scraper.

In case a longer maintenance was desired the injected corneas were excised and washed by dipping them three times into a beaker of physiologic saline. The excess fluid was drained off, and the corneas were placed on filter paper (epithelium upwards) for a more complete drainage of excess fluid, and covered with a petri dish for protection. Adherent drops of fluid were removed by cautiously touching the rim of the cornea with a filter paper. Contact between epithelium and filter paper to any greater extent should be avoided. The corneas were then transferred to a watchglass with the epithelium upwards, trapping, if possible, an air bubble, so as to avoid contact between the corneal endothelium and the glass, and maintaining the natural convexity of the tissue. As many as 30 corneas were placed on one 16 cm. watchglass.

The watch glass was placed in a glass jar (20 cm. in diameter, 20 cm. in height) with a well fitting lid. The proper humidity can be obtained by placing several layers of wet paper towels under the lid. At a proper degree of humidity the corneas should neither be dehydrated to such an extent that the rims stick to the glass, nor should water of condensation form on the surface of the epithelium. This latter precaution is of great importance for a satisfactory maintenance. We observed that any free fluid, especially on the upper epithelial surface of the cornea, greatly increases the incidence of bacterial contamination, which is the main danger to the successful maintenance during a longer incubation period. Fortunately very small bacterial colonies can be detected as little gray spots on the transparent tissue. Infected corneas were discarded. The resistance of the uninjected cornea to bacterial contamination must be considerable. Working without special precautions of sterility, infection was rarely observed even after incubation for 20 hours at 32°C. and it was extremely rare at an incubation temperature of 29°C. The incidence of infection was somewhat more frequent with injected corneas. However, the use of sterile instruments, sterile wash fluid and glassware, and face masks during the preparation of the cornea decreased the incidence of infection.

The adhesion of the epithelium in uninjected corneas remains practically unaltered during incubation at 29°C. for 20 hours. Increase of the incubation temperature above 35°C. decreases the adhesion slightly and promotes the development of bacterial colonies. The adhesion of the epithelium in corneas with injected fluid in the stroma is more affected by incubation. In about one third of the samples the critical pressure drops to 80–100 grams. However, even in these samples the amount of epithelium removed at 40 grams is always less than 35%. It is advisable to use rather large numbers of corneas in each experiment, and to determine the effect of the tested substances not with a single dose but preferably by defining the highest ineffective and the lowest effective concentration, and to carry along a sufficient number of controls. The controls are injected with a saline solution of approximately the same tonicity as the solution injected into the experimental samples. If such precautions cannot be applied, an effect should not be regarded as significant unless more than 70% of the epithelium is removed at a pressure of 40 gms.

RESULTS

With the method described in the preceding paragraphs, we tested the influence of various experimental factors upon the adhesion of the epithelium.

1. *Temperature.* The corneas were exposed for different lengths of time to a wide range of temperatures, and the effect was tested immediately after termination of the exposure, and also after a subsequent incubation period of 20 hours at 29°C.

The effect of higher temperatures was tested by placing the corneas in warmed solutions of 0.9% NaCl. At 52°C. the tissue becomes lacy and the epithelium is rapidly loosened. Within ten minutes the critical scraping pressure drops to below 40 grams. Exposure to 45°C. for half an hour produced no immediate effect and none was observed on subsequent incubation at 29°C. However, continuous exposure to 45°C. for many hours produces a gradual loosening of the epithelium.

For the exposure to low temperatures, the corneas were put into petri dishes. No fluid was added but the dishes were covered with wet paper towels in order to maintain adequate humidity. The petri dishes were kept for the desired length of time in the main

compartment of a refrigerator at -2°C . or in a freezing unit at -15°C . After exposure for one hour to -2°C . no effect could be noticed. After freezing of the corneas at -15°C . and subsequent thawing, the critical scraping pressure was found to be normal, but with subsequent incubation for 10 hours at 29°C . marked loosening of the epithelium developed (Table I).

2. *Hydrogen Ion Concentration and salts.* Corneas were bathed for one hour in physiologic saline, the pH of which was adjusted (color indicator) to a desired value by addition of N/1 HCl or NaOH. Glycine buffer of equal molarity was used instead of saline for the establishment of pH 11 and 12. Three to five corneas were used for each test. The pH of the solutions shifts slightly within the first

TABLE I
Effect of Temperature on Adhesion of the Epithelium

TEMPERATURE IN $^{\circ}\text{C}$.	TESTED IMMEDIATELY		TESTED AFTER INCUBATION AT 29°C . FOR 20 HOURS	
	Pressure in grams	% Epithelium removed	Pressure in grams	% Epithelium removed
52° for 10 min.	40	98, 96		
45° for 30 min.	95	30	95	20, 25
-2° for 60 min.	95	21	95	20, 49
-15° for 15 min.	95	35	40	98
-15° for 30 min.	95	28	40	95

half hour due to the buffer-capacity of the corneas and has to be readjusted. After one hour the corneas were removed from the bath, drained briefly and tested with the scraper.

Over a wide range of the pH scale no significant effect was observed (Table II). In strongly acid and alkaline solutions much epithelium was removed at a pressure of 95 grams, but these results were due to its complete disintegration rather than to a removal at the boundary. At pH 12 the epithelium is transformed into viscous strands, and at pH 1 only the superficial portion of the epithelium was removed leaving a considerable extent of the stromal surface covered by the basal layer. The disintegration of the epithelium at low pH seemed to be determined not only by the concentration of hydrogen ions, but also by the ionic strength as such, for we observed regularly that the

epithelium came off as a whole at a pressure of 40 grams when the N/10 solution of HCl was made up with distilled water (Table III) instead of saline. The adhesion was normal or even slightly greater than normal when the hydrochloric acid in the bathing fluid was replaced by acids which are characterized by their protein precipitating capacity (5, 6).

TABLE II
Influence of pH on Adhesion of the Epithelium

pH	PRESSURE IN GRAMS	% EPITHELIUM REMOVED
1.0	95	55, 67, 62
2.0	95	29, 44, 33
3.5	95	42, 22, 36
4.5	95	11, 14
6.3	95	18
7.0	95	20
9.0	95	15, 12
11.0	95	91
	60	43, 38
12.0	60	77, 80

TABLE III
The Effect of Various Protein Precipitating Acids on the Adhesion of the Epithelium

REAGENTS USED 0.1 M.	PRESSURE IN GRAMS	% EPITHELIUM REMOVED
Tannic Acid	165	12
	200	66
Sulfosalicylic Acid	165	42
Trichloroacetic Acid	165	48
Metaphosphoric Acid	200	53
Tungstic Acid	165	59

Neutral solutions of most of the salts tested were without significant effect. Salts of the alkaline earth caused some decrease of the adhesion, but the effect could be recognized only if the excess of these ions were removed by a prolonged washing of the corneas in saline. Thiocyanate and urea were also somewhat effective (Table IV). Removal of calcium either by prolonged washing with saline or by suspending the corneas in N/5 oxalate solutions for one hour did not alter the adhesion (Table V).

3. *Alcohols.* A series of aliphatic alcohols was compared by bathing the cornea for one hour in solutions of these compounds in 0.9% saline. The members of this series with the longer carbon chains were far more effective than the homologues with a small number of carbon atoms. Polyalcohols such as glycerol and sucrose were ineffective. In fact, on adding glycerol to butyl alcohol, the loosening

TABLE IV

Effect of Solutions of Various Substances on the Adhesion of the Epithelium
Corneas placed for 1 hour in M/1 solution at pH 7

INEFFECTIVE		LOSS OF ADHESION
Sodium Chloride	Sodium Sulfate	Calcium Chloride
Potassium Chloride	Sodium Bisulfite	Barium Chloride
Potassium Iodide	Sodium Thiosulfate	Strontium Chloride
Sodium Fluoride	Sodium Nitrate	Potassium Thiocyanate
Potassium Bromide	Disodium Phosphate	Urea
Sodium Acetate	Mono Sodium Phosphate	
Glucose	Ferric Chloride	
Sodium Citrate	Magnesium Sulfate	
Sodium Oxalate	Copper Sulfate	
Glycerol	Lanthanum Nitrate	

TABLE V

Adhesion of the Epithelium after Bathing of the Corneas for 1 hour in Solutions of Various Ions

REAGENTS USED	PRESSURE IN GRAMS	% EPITHELIUM REMOVED
Calcium Chloride, 1.0 M, pH 6.5	95	95
	40	51
Lithium Sulfate, 1 M, pH 6.8	95	21, 9
Sodium Oxalate, 0.2 M, pH 6.8	95	11, 13

effect of the latter could not be detected if tested immediately after the treatment. However, this antagonistic action disappeared, and a complete loss of adhesion occurred if the samples were washed with saline and the excess of glycerol was removed. Glycerol was ineffective as an antagonist if it was applied, not simultaneously, but subsequently to the treatment with butyl alcohol (Table VI).

4. *Butyl derivatives.* In view of the efficacy of butyl alcohol, we tested some other derivatives of the butyl radical. Butyric acid and butylamine in 1M concentration are ineffective at a pH of 6.5. How-

TABLE VI
Effect of Alcohols on Adhesion of the Epithelium

REAGENT USED	PRESSURE IN GRAMS	% EPITHELIUM REMOVED
Methyl alcohol 10 M	95	18
Ethyl alcohol 10 M	40	65
5 M	95	63
2.5 M	95	24
Propyl alcohol 5 M	40	88
2.5 M	40	81
1.2 M	95	25
Butyl alcohol 0.5 M	40	98
0.25 M	95	15
0.12 M	95	11
Amyl alcohol 0.2 M	40	92
0.1 M	95	17
Glycerol 1 M	95	16
Glycerol 1 M +		
Butyl alcohol 0.5 M (tested immediately after treatment)	95	43, 34
Glycerol 1 M +		
Butyl alcohol 0.5 M (tested after washing in saline for 1 hour)	40	95, 87

ever, at pH 2 butyric acid is somewhat more effective than the alcohol, and at pH 4.5 it has a slight effect. Butyl amine is ineffective up to pH 8.5. At still higher pH butyl amine becomes insoluble.

An enhancement of the efficacy of butyl alcohol by the introduction of a chlorine atom is seen in comparing butanol with chlorobutanol, and ethanol with ethylene chlorhydrin. Another related compound, chloral hydrate, is somewhat less effective as a loosening agent.

The ether of butyl alcohol with ethylene- or diethylene glycol is as effective as butyl alcohol. Polyglycols themselves up to a chain length of nine carbons have no loosening effect but, on the contrary, they seem to increase the cohesion of the tissue.

5. *Detergents.* Butyl alcohol and the derivatives tested contain both hydrophilic and hydrophobic groups assymmetrically arranged in the same molecule. We have extended our tests to compounds in which this assymetry is even more pronounced. Such substances are generally classified as detergents. Mannitan laurate was used as an example of a neutral detergent. The hydrophilic group in this compound is a poly-alcohol. In concentrations up to 1M it had no effect on the adhesion of the epithelium to the stroma.

In the anionic detergents which we used, the hydrophilic group is a sulfate or sulfonate radical. These substances do not loosen the epithelium from the stroma but gradually transform the epithelium into a slimy mass. The process starts in the superficial layers of the epithelium. If it is interrupted at an early stage, the basal layer is found still intact and firmly adherent. This phenomenon seemed unrelated to that which we were studying and was not investigated further. The lowest concentration at which this effect was observable was found to be between 0.1 and 0.01 M.

In the cationic detergents the hydrophilic group is a quaternary ammonium or pyridinium radical. Three of the four substances in this group which we tested caused a loosening of the epithelium. With the substances the effect was somewhat different from that produced by butyl alcohol. The epithelium became hazy and fragile and did not peel off in a coherent sheet. With the lower concentrations the effect seemed to be confined to the superficial layer of cells. The lowest effective concentration was about 0.005M (Table VII).

6. *Enzymes.* Solutions of the various enzymes were injected into beef corneas and the tissues incubated at 23–24°C. for 4 hours or more, after which the adhesion of the epithelium to the stroma was tested. Recrystallized samples of trypsin and chymotrypsin were

kindly supplied by Dr. R. Herriott (Rockefeller Institute, Princeton). They were obtained in the form of ammonium and magnesium sulfate

TABLE VII

Effect of Various Substances on the Adhesion of the Corneal Epithelium

	LOWEST MOLAR CONC. EFFECTIVE IN LOOSENING EPITHELIUM	HIGHEST MOLAR CONC. WHICH FAILED TO LOOSEN EPITHELIUM
A. Alcohols and Related Compounds		
Butanol	0.5	0.2
Butyrate		
pH 2	0.3	0.1
pH 4.5	1.0	0.5
pH 6.5		1.0
Butylamine		
pH 7		1.0
pH 8.5		1.0
Chlorobutanol	0.06	0.03
Ethylenchlorhydrine	0.5	0.25
Chloralhydrate	1.2	0.8
Butylcellosolve	0.5	0.2
$\text{CH}_3(\text{CH}_2)_3\text{OCH}_2\text{CH}_2\text{OH}$		
Butylcarbitol	0.5	0.2
$\text{CH}_3(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{OH}$		
Diethyleneglycol		3.0
$\text{OHCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH}$		
Nonaethyleneglycol		3.0
$\text{HO}(\text{CH}_2\text{CH}_2\text{O})_9\text{H}$		
B. Neutral Detergents		
Mannitan laurate		1.0
Mannitan lauric acid ester Atlas Co.		
C. Cationic Detergents		
Zephiran (Alba Pharmaceutical Co.)	0.005	0.002
Alkyl dimethyl benzyl ammonium chloride (alkyl = C_8 — C_{18})		
Triton K-60 (Röhm and Haas)		0.2
Lauryl dimethyl benzyl ammonium chloride		
Emulsol 607	0.005	0.002
Lauric acid ester of colamino-formyl methyl pyridinium chloride		
Emulsol 607 M (Emulsol Co.)	0.005	0.002
Myristic acid ester of colaminoformyl methyl pyridinium chloride		

TABLE VII—*Continued*

	LOWEST EFFECTIVE CON- CENTRATION PRODUCING LIQUEFACTION (MOLAR)	HIGHEST MOLAR CONC. WHICH FAILED TO LOOSEN EPITHELIUM
D. Anionic Detergents		
Duponel (E. I. duPont de Nemours)	0.005	
Sodium lauryl sulfate		
Drene (Proctor and Gamble)	0.005	
Triethanolamine lauryl sulfate		
Igepon AP (General Dyestuffs)	0.005	
R—COO—(CH ₂) ₂ SO ₄ Na		
Triton W-30 (Röhm and Haas)	0.005	
Sodium salt of alkyl phenoxy ethyl sulfonate		
Tergitol-7 (Carbon and Carbide Corp.)	0.005	
C ₄ H ₉ ·CH·(C ₂ H ₅)·C ₂ H ₄ ·CH(SO ₄ Na)- C ₂ H ₄ CH(C ₂ H ₅) ₂		
	LOWEST MOLAR CONC. EF- FECTIVE IN LOOSENING EPITHELIUM	
E. Enzymes		
Trypsin	5-10 mg./cornea	0.5 mg./cornea
Chymotrypsin	5-10 mg./cornea	0.5 mg./cornea
Hyaluronidase		1 mg./cornea
Ribonuclease		1 mg./cornea
Pancreatic lipase		Activities see text

cakes. Solutions of these preparations were dialyzed against running tap water for 6 hours and then overnight in the icebox against 0.9% saline. The protein content was determined by precipitating the protein in an aliquot of the dialyzed sample with trichloroacetic acid and determining the nitrogen content in the thoroughly washed precipitate. A recrystallized preparation of ribonuclease was also obtained from Dr. R. Herriott. A purified and highly active sample of hyaluronidase prepared from bull testis was supplied by Dr. K. Meyer, Columbia University. Pancreatic lipase was prepared and purified by Dr. M. Bovarnick (Department of Biochemistry, Johns Hopkins Medical School). The amounts of this preparation which were used for injection hydrolyzed triacetin equivalent to 0.7 ml. of N/10 NaOH and tributyrin equivalent to 3.9 ml. N/10 NaOH per hour at 37°C. The enzyme was free from proteolytic activity. From

Table VII it can be seen that among these enzymes only trypsin and chymotrypsin cause a decrease of the adhesion under our experimental conditions.

7. *Inhibition of metabolism.* The role of oxygen for the maintenance of the tissue cohesion was assayed by incubating the corneas under anaerobiosis. A desiccator was used as moist chamber for the preservation of the cornea. After placing the corneas inside, the vessel was evacuated for ten minutes to a pressure of 50 mm. Hg. Afterwards the desiccator jar was placed in an incubator and connected with a tank of nitrogen or a mixture of 95% N + 5% CO₂. The gases were purified by passing through an alkaline pyrogallol solution or over copper wire at 500°C. A gentle flow of the gas was maintained

TABLE VIII

Adhesion of the Epithelium after Injection of Cyanide and Malonate and Incubation at 29°C.

REAGENT INJECTED PER CORNEA pH 7.0	INCUBATION TIME HOURS	PRESSURE IN GRAMS	% EPITHELIUM RE- MOVED
Sodium Cyanide	12	95	31
1 mg. (approximately 0.02 M)	20		25
5 mg. (approximately 0.1 M)	12	95	12
	20		15
Sodium malonate			
2 mg. (approximately 0.08 M)	20	95	25, 35

throughout the incubation period. After anaerobic incubation for 20 hours the corneas were perfectly clear and the epithelium showed the same adhesion as the controls. Incubation for 10 hours anaerobically and for the subsequent 10 hours aerobically was likewise without effect. The insensitivity of the adhesive mechanism to the availability of oxygen is demonstrated also by another experiment in which cyanide was injected in amounts up to 5 mg. per cornea, equivalent to a concentration of approximately 0.1 M. Again no sign of loosening was detectable, and malonate (which is supposed to inhibit succinyldehydrogenase) was also without influence (Table VIII).

On the other hand a great decrease of the adhesion can be observed after injection of iodoacetate and fluoride (Table IX and Fig. 4)

TABLE IX

Effect of Iodoacetate and Fluoride on the Adhesion of the Epithelium

REAGENTS INJECTED	INCUBATION AT 32°C. FOR 12 HOURS		INCUBATION AT 29°C. FOR 20 HOURS	
	Pressure in grams	% Epithelium removed	Pressure in grams	% Epithelium removed
Fluoride, 0.3 mg. per cornea (approximately 0.007 M)	40	90	40	87, 92
	40	50	40	49
	60	76	60	61
	40	28, 20		
	60	17	60	18, 37
Iodoacetate, 0.03 mg. per cornea (approximately 0.001 M)	40	96, 93	40	61
	60	35	40	
	95	56	60	14
	60	22	60	28
	95	31		

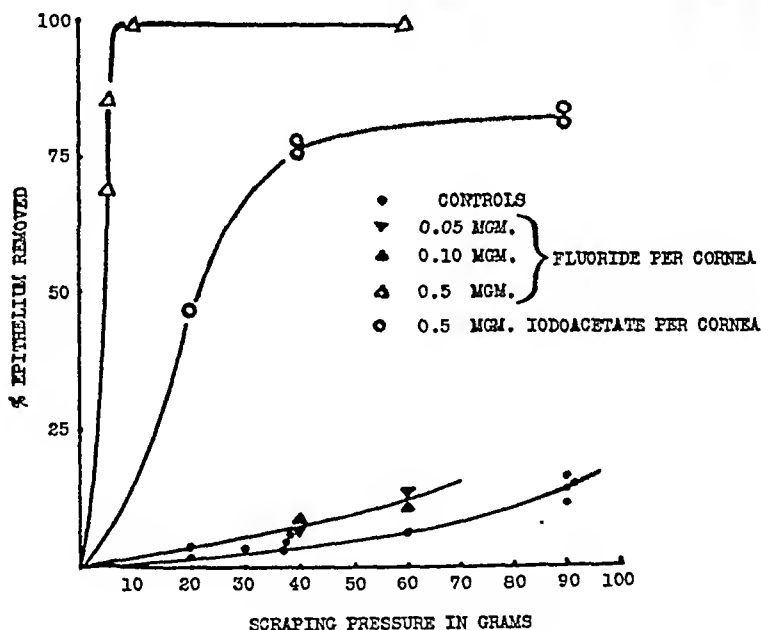


FIG. 4 EFFECT OF IODOACETATE AND FLUORIDE ON THE ADHESION OF THE CORNEAL EPITHELIUM

which are known primarily as inhibitors of reactions in the glycolytic pathway. The effect in respect to the loosening of the epithelium developed after a lag period of about 6–10 hours. At lower incubation temperatures the effect decreased markedly (Table X).

DISCUSSION

a. *Method*: Our method for the measurement of the adhesion of the epithelium of the corneas has been developed on a purely empirical basis. Closer analysis shows that the connection between our figures and the adhesive forces is an indirect one, as may be seen from the following simplified scheme of the process of separation.

Adhesion of epithelium to stroma is here defined as the force with which the epithelium resists mechanical removal from the stroma. For a direct determination of the adhesion it would be necessary to measure the forces which are required to pull the epithelium from the stroma. Such a force could be applied either in a direction vertical

TABLE X

The Effect of Incubation and Temperature upon the Loss of Adhesion Caused by the Injection of Fluoride and Iodoacetate

REAGENT INJECTED PER CORNEA	18°C.		29°C.	
	Pressure in grams	% Epithelium removed	Pressure in grams	% Epithelium removed
Sodium fluoride 0.12 mg.	40	4, 17, 22	40	96, 87, 81
	60	21, 16		
	80	26		
0.25 mg.	40	33, 24	40	79, 80
Sodium Iodoacetate 0.5 mg.	40	10, 33	40	73, 89
	60	19, 29	40	85, 84
	95	23, 57	40	88, 87

to the surface of the epithelium, or as a shearing force parallel to the stroma surface. As long as the adhesion of the epithelium is normal, or only moderately decreased, it is hardly possible to remove any epithelium by pulling vertically to the corneal surface. Only when the tissue has completely lost its cohesion can the epithelium be pulled off in large flakes or in one whole sheet, (e.g. after bathing in butyl alcohol or after incubation with higher doses of iodoacetate or fluoride). Therefore, only the second alternative seemed practicable. If the epithelial edge, towards which the pushing force of the scraper is directed, stands perpendicular to the surface of the stroma, the values obtained for this force should be a direct measure

of the adhesive forces (Fig. 5). Actually the contact angle between stroma and epithelium will be greater than 90° . In this case the force with which the epithelium will counteract the pressure of the scraper (R') will be represented by two components (see Fig. 5). The one (a) counteracting the horizontal force of pressure, the other (b) acting on the blade in vertical direction. By splitting R' into two components (a and b) two magnitudes should become available as indices of the adhesion. The possibility of measuring component (a) by determining the force which is necessary to pull the scraper

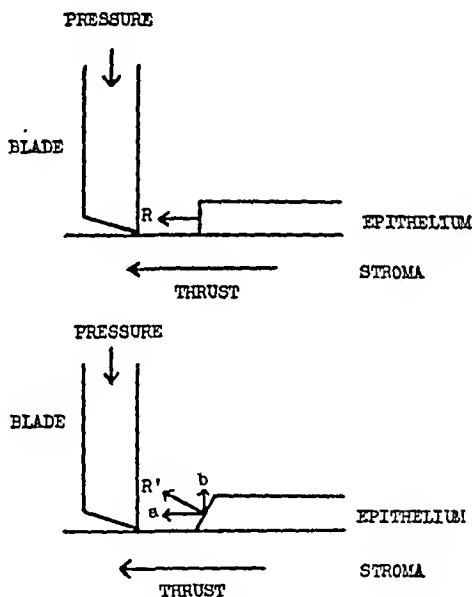


FIG. 5 FORCES INVOLVED IN SCRAPING OFF EPITHELIUM

over the surface of the stroma eliminating by a suitable weight the action of component (b) seems hardly feasible since the friction on the stroma surface would be of a comparable order of magnitude. In our procedure component (b) is measured by determining the weight which is required to keep the blade on the surface of the stroma while the epithelium is removed. An uncontrolled variable in determining component (b) is the contact angle between epithelium and stroma. This angle will depend upon the turgescence and general condition of the epithelial cells. Our practical experience shows, however, that these variables do not interfere with the results of our relatively

crude measurements. We observed the same difference in the adhesion between experimental samples and controls in direct measurements, and after fixation of the corneas in 5% trichloroacetic acid, during which differences in the state of the epithelial cell and its turgescence should have been eliminated. We were unable to detect a difference in the water content (as index of turgescence) of control and experimental samples after iodoacetate injection which caused a complete loss of the adhesion of the epithelium. Although it appears that the differences in our measurements indicate actual differences in the cohesion of the tissues, turgescence and the other factors which influence the contact angle would have to be considered if smaller variations in the adhesion than those which are described in our experiments were to be measured.

b. *Results:* With our experiments we attempted to obtain some information about the physical or chemical basis of the adhesion of the epithelium to the stroma. The aggregation of single cells in multicellular organisms requires either the presence of an enveloping extracellular coat, or cohesion of the cells to one another. These two principles have been sharply contrasted in an extensive study by Holtfreter (7) who pointed out that the cells are bound together by an external coat at early stages of the embryonic development, whereas the cell surface proper becomes the site of cohesion at more advanced stages of differentiation. An instance where a more or less specific intercellular cement is supposed to provide the connecting element between cells is the capillary endothelium (8). Great significance has been attributed to this substance in relation to the fragility of the capillary wall.

The chemical qualities of the external coat are badly defined, and only a few of its properties have become known (9). It is greatly altered by a change of the pH from 6.4 to 8.0, and is stable only in the presence of calcium ions. Experiments with these agents failed to loosen the epithelium of the cornea. The removal of calcium by washing the cornea with oxalate solution has no noticeable influence, and the adhesion remains unchanged over the greater part of the pH scale. Therefore, it seems unlikely that the epithelium is attached to the stroma by a material which is similar to the external coat of developing eggs or to the intercellular cement of the capillary wall.

It would seem more likely that the adhesion in the case of the cornea epithelium is a property of the cell surface itself, or of the surface of the collagen bundles of the stroma.

The stroma has a very high content of a mucopolysaccharide and experiments were carried out to ascertain the role of this substance as a possible factor for the adhesion of the epithelium to the stroma. The mucopolysaccharide of the cornea has been isolated and analyzed by Meyer and Chaffee (10) who found it to contain one sulfo group per molecule. Sulfonated mucopolysaccharides are acids with great affinity to the positively charged groups of the protein molecule. At low pH these mucopolysaccharides form insoluble salts with proteins and these salts are not decomposed by comparable concentrations of anions with lower protein affinity, e.g. chloride (11). However, on addition of anions with greater or equal affinity an exchange reaction takes place, the characteristics of which are determined by the relative affinities and concentrations of the competing ions. On the alkaline side of the isoelectric point of the protein component the salts undergo dissociation.

As a first step, we attempted to test the possibility that such an acid base linkage across the stroma epithelium barrier might be the basis for the cohesion of the tissues. Our experiments, however, do not support this assumption. The following data are relevant in this connection. (a) In the alkaline range loosening of the epithelium does not occur below pH 10. In order to relate this finding to the hypothetical acid-base cohesion one would have to assume that adherent protein has its isoelectric point about pH 10. This is unlikely since only the proteins of nucleus are known to have this property. (b) If the hypothesis were correct, loosening of the epithelium should occur in the presence of anions with strong protein affinity. In contrast, we found that such ions cause a slight increase in the adhesion of the tissue. (c) Our only experimental finding which would seem to be in agreement with the above assumption is the loosening of the epithelium in the presence of high concentrations of calcium salts, since calcium ions have apparently a considerable dissolving action on mucopolysaccharides. However, calcium ions have so many affinities with tissue components that no specific interpretation can be offered to explain this finding. It should be pointed out that calcium

has, e.g., a high affinity to cephaline in monomolecular layers (12). (d) The failure to obtain any effect on epithelial adhesion by any other of the many ions we have tested also speaks against the hypothesis of an acid base linkage between stroma and epithelium. (e) The participation, in particular, of strongly ionized substances in the cornea, such as mucopolysaccharides or nucleic acids is made unlikely by the negative results with hyaluronidase and ribonuclease, the enzymes which are supposed to destroy these compounds.

These negative results are discussed in detail because the rejection of an ionic linkage as the basis of the cohesion compelled us to consider possible non-ionic forces. In experiments with alcohols the efficacy was found to increase with increasing length of their aliphatic chains. The effect of some alcohols is enhanced by the further development of the hydrophobic portion of the molecule, as for example in chlorine substituted compounds. Amplification of the hydrophilic group, on the other hand, does not enhance the effectiveness, in fact polyalcohols operate in the reverse direction, increasing the adhesion of the epithelium and suppressing the loosening effect of butanol if added simultaneously to the corneas.

These findings could be interpreted by assuming that a lipid layer is of significance in the establishment of the adhesive mechanism. The presence of lipoids in the surface layer of cells is well established. A detailed picture of the molecular pattern of the cell surface has been proposed by Danielli and Harvey (13) and has been elaborated on by Danielli and Davson (14) and by Danielli (15). The experimental basis for the hypothesis of these authors was obtained by measurement of the surface tension of marine eggs and other mon-cellular organisms. According to these authors the cell surface can be described as a double layer of lipid molecules, their hydrophobic tails oriented towards each other, and a layer of protein adsorbed by their hydrophilic heads. The structure of such a multilayer should be markedly disturbed by the addition of long chain alcohols. Thus, for example, it may be supposed that the alcohols are absorbed in the lipid double layer as the result of the lipid affinity of their hydrocarbon chains, while at the same time introducing an aqueous phase between the two sheets of the lipid double layer. A possible representation of such a disruption can be found in a paper by Schmitt

(16). In suggesting this as a possible explanation for the loosening of the epithelium by alcohols we are assuming that the boundary between epithelium and stroma consists of a molecular array similar to that suggested by Danielli and co-workers for the individual cell of unicellular organisms. In connection with the antagonistic effect of glycerol and butyl alcohol on the loosening of the epithelium an interesting observation, mentioned in the discussion (p. 24) of an article by Parpart and Dziemian (17), should be referred to. These authors found that the penetration of glycerol into red blood cells of some species is accelerated by butyl alcohol and in other species suppressed. It should be pointed out that a disruption of a multilayer, such as is described above, could take place at different levels. In all cases the phenomenon of the loss of adhesion will appear the same, as long as no finer means of differentiation become available. Some evidence on this point can be seen in the fact that injection of a solution of recrystallized trypsin and chymotrypsin causes a loosening of the epithelium which indicates the presence of a protein in the coherent multilayer. An interpretation of the adhesion of the epidermis of the skin based entirely upon the properties of the collagen of the dermis has been advanced in a recent publication by Felsher (18).

Whatever the molecular arrangement which has to be regarded as the direct cause of the cohesion, it is remarkable that aerobiosis is not required for the maintenance of the adhesion of the epithelium, as demonstrated in experiments under anaerobiosis and after injection of cyanide. On the other hand the dependence on anaerobic metabolic processes is suggested by the sensitivity of the adhesion to iodoacetate and fluoride, two metabolic poisons known to interfere with the anaerobic phase of the carbohydrate metabolism. An analysis of the present status of our knowledge of the metabolism of the cornea will be given in one of the following papers. As an example of other cellular activities, involving the cell surface, which are maintained under anaerobiosis and impaired by iodoacetate and fluoride, mention should be made of the phagocytic activity of leucocytes as discussed in an article by Fleischmann (19, pp. 291-292).

c. *Relation to vesicants*: Loosening of the corneal epithelium is a phenomenon which invites comparison with that of vesication in the skin. In addition to the effects of various chemical and physical

agents reported in this paper it may be pointed out that bacterial contamination causes a loss of cohesion in the corneal tissues supravitaly, and that infection and inflammation of the cornea lead to loosening of the epithelium *in vivo*. Loosening of the epithelium after exposure to ultraviolet light is a well known clinical phenomenon and has been observed experimentally (20). For the present, however, we wish merely to point out that the analogies are not complete.

Experiments by Danielli (21) on the vesication in frogs' skin in which the effects of various metabolic poisons were explored revealed some significant contrasts in comparison to our results in the cornea. For instance, cyanide and azide fail to loosen the corneal epithelium, but cause loosening of the epithelium in the frog's skin. On the other hand, iodoacetate is a powerful agent in loosening the corneal epithelium but was found ineffective on the frog's skin by Danielli. These differences may be due to differences in the species or in the tissues studied, and indicate that the metabolic pathways by which energy is supplied for the maintenance of similar aspects of tissue organization may differ in different tissues or in different species.

SUMMARY

1. A simple mechanical device is described for the quantitative estimation of the adhesion of the epithelium to the corneal stroma.
2. Exposure of the tissue to temperatures over 45°C. or freezing and subsequent incubation lead to a loosening of the corneal epithelium.
3. No loosening of the epithelium was observed on altering the pH over the range pH 3 to pH 9, on removal of calcium, on addition of protein precipitating agents, or on exposure to a wide variety of inorganic salts. Exceptional among the latter were sodium thiocyanate and high concentrations of calcium chloride, both of which caused loosening of the epithelium.
4. Exposure of the tissue to aqueous solutions of alcohols in low concentration markedly decreased the tissue cohesion. The minimum effective concentration decreased with increasing molecular weight of the alcohols, amyl and butyl alcohol and some chlorine substitutes of the latter were the most effective agents of this type so far tested.

(16). In suggesting this as a possible explanation for the loosening of the epithelium by alcohols we are assuming that the boundary between epithelium and stroma consists of a molecular array similar to that suggested by Danielli and co-workers for the individual cell of unicellular organisms. In connection with the antagonistic effect of glycerol and butyl alcohol on the loosening of the epithelium an interesting observation, mentioned in the discussion (p. 24) of an article by Parpart and Dziemian (17), should be referred to. These authors found that the penetration of glycerol into red blood cells of some species is accelerated by butyl alcohol and in other species suppressed. It should be pointed out that a disruption of a multilayer, such as is described above, could take place at different levels. In all cases the phenomenon of the loss of adhesion will appear the same, as long as no finer means of differentiation become available. Some evidence on this point can be seen in the fact that injection of a solution of recrystallized trypsin and chymotrypsin causes a loosening of the epithelium which indicates the presence of a protein in the coherent multilayer. An interpretation of the adhesion of the epidermis of the skin based entirely upon the properties of the collagen of the dermis has been advanced in a recent publication by Felsher (18).

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SUMMARY

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2. Exposure of the tissue to temperatures over 45°C. or freezing and subsequent incubation lead to a loosening of the corneal epithelium.

3. No loosening of the epithelium was observed on altering the pH over the range pH 3 to pH 9, on removal of calcium, on addition of protein precipitating agents, or on exposure to a wide variety of inorganic salts. Exceptional among the latter were sodium thiocyanate and high concentrations of calcium chloride, both of which caused loosening of the epithelium.

4. Exposure of the tissue to aqueous solutions of alcohols in low concentration markedly decreased the tissue cohesion. The minimum effective concentration decreased with increasing molecular weight of the alcohols, amyl and butyl alcohol and some chlorine substitutes of the latter were the most effective agents of this type so far tested.

On the other hand polyalcohols such as sucrose or glycerol failed to loosen the epithelium.

5. The cohesion of the tissue is well maintained on anaerobic incubation and in the presence of cyanide and of malonate. Loosening of the epithelium occurs on incubation of tissue exposed to iodoacetate or fluoride.

6. Exposure to proteolytic enzymes causes a loosening of the epithelium.

7. These studies reveal no evidence in favor of the presence in the cornea of an intercellular cement similar to that characteristic of the capillary wall. Furthermore, they indicate that the cohesive force is probably not due to electrostatic attractions of oppositely charged ionic groups. On the contrary, the experiments suggest that the adhesive forces originate in the attraction of non-polar hydrocarbon residues in the boundary surface, and that the coherent surface may be a protein lipid multilayer. In attributing the adhesive force to hydrocarbon residues lipoids may be considered as the most likely source, but the affinities of non-polar side chains of proteins such as collagen are not excluded at the present. The maintenance of the coherent organization may require metabolic energy, since the cohesion is lost on iodoacetate and fluoride poisoning.

8. The phenomenon of epithelial loosening in the cornea bears certain obvious analogies to that of vesication in the skin. Some dissimilarities between the two processes have, however, already been revealed.

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VIII. THE EFFECT OF HISTAMINE AND RELATED SUBSTANCES ON THE COHESION OF THE CORNEAL EPITHELIUM*

HEINZ HERRMANN

In the preceding paper we described the use of a simple mechanical scraper with which it is possible to remove the epithelium of the excised cornea and to obtain at the same time a crude quantitative estimate of the adhesive forces. A decrease of the cohesion was observed when the corneas were bathed in weak solutions of higher aliphatic alcohols (butyl, amyl) and of some of their chemical derivatives, and a loosening was also observed on incubating for about 10 hours after injection into the cornea of some metabolic poisons such as iodoacetate or fluoride. These results made it seem desirable to find out whether the neurohormones and commonly used drugs produced similar effects. Too few compounds have been examined to allow for a systematic evaluation. However, we found that histamine differed in its effects from all the other drugs investigated so far, both qualitatively and quantitatively.¹ The results are published as a contribution to the pharmacology of histamine, since a continuation of the examination of other drugs is not planned at the present time.

RESULTS

The experiments were performed on beef corneas. The maintenance of the corneas, the supply of drugs, and the method for the determination of the adhesion have been described in the preceding paper. The adhesion of the epithelium was defined by the amount of epithelium removed as a function of the weight of the scraper. In fresh normal corneas very little epithelium (less than 30%) is removed at a pressure of 90 gms., and a weight of 200 gm. is required for complete removal. The greater the loss of cohesion, the smaller the weight

*The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

¹ With the exception of colchicine which causes loosening in equally low concentrations.

which is required for the removal of a certain amount of epithelium. When corneas are injected with saline, the cohesion decreases somewhat on incubation, corresponding to a range of weights of 60 gms. to 120 gms. for the minimum and maximum removal respectively. When 1 microgm. of histamine is injected into the cornea most of the epi-

TABLE I

The Effect of Histamine and Other Natural Bases on the Loosening of the Epithelium
Incubation: 20 hours at 29°C.

CONTROLS		REAGENT INJECTED	AMOUNT INJECTED PER CORNEA	PRESSURE IN GM.	% EPITHELIUM REMOVED	
Pressure in grams	% Epithelium removed					
60	14, 20	Histamine	10 micrograms	40	90, 89	
				40	92, 92	
			5 micrograms	40	86	
			3 micrograms	40	85	
45	45		1 micrograms	40	85, 66	
					98, 61	
			0.3 micrograms	60	42	
				40	23	
		Histidine	2 mg.	95	35	
			1 mg.	60	25, 11	
		Arginine		95	45	
			1 mg.	60	12, 32	
60	28, 25				9, 18	
					8	
			Asparagine	1 mg.	60	19, 17
			Tyramine	1 mg.	95	48, 28
			0.5 mg.	95	17	
			0.1 mg.	95	25, 28	
			Creatine	1 mg.	95	29
			Creatinine	1 mg.	95	23
95	8	Putrescine	0.5 mg.	95	18	
60	11	Arginine +	1 mg.	40	91, 95	
		Histamine	10 micrograms		67	

thelium can be removed with a pressure of 40 gms., the lowest weight available on the scraper (Table I). The effect of histamine does not develop immediately but requires an incubation period of about 8 hours (Table II). None of the other substances tested which are chemically related to histamine, such as histidine, tyramine, and other natural bases, show any effect at all. Some of the other pharma-

cologically active compounds, adrenaline, ephedrine, physostigmine, however, cause a loosening, but the concentrations which are required are considerably higher than in the case of histamine. Whether the loosening action of high concentrations of physostigmine is due to its parasympathetic properties is doubtful since acetylcholine, as such or in the presence of low concentrations of physostigmine, has no loosening effect. A loss of adhesion after prolonged application of high doses of pontocaine and other local anaesthetics has been frequently observed clinically.

Histamine is remarkable not only because of the small quantities which suffice to cause a loosening of the epithelium, but also because it differs from the other effective substances in the mode of its action. If compounds which decrease the adhesion of the epithelium are in-

TABLE II

The Development of the Effect of Histamine during Incubation at 29°C.

10 Micrograms Histamine per cornea.

INCUBATION TIME IN HOURS	PRESSURE IN GRAMS	% EPITHELIUM REMOVED
1	60	10, 18, 8
4	60	5, 21, 19
10	60	55, 22, 74
20	40	82, 90

jected in a proper dose, the loss of the adhesion can develop to such an extent that it becomes possible to remove the epithelium as a coherent sheet. If this operation is carried out after injection of fluoride or iodoacetate no epithelium remains on the surface of the stroma. After the injection of histamine a very thin film remains on the surface of the stroma which adheres firmly. This film consists of a single cell layer which remains on the stroma surface even after scraping with a pressure up to 60 gms. In histological preparations in which the epithelium was not removed after histamine injection, one can observe more frequently than in normal samples a fine discontinuity between the basal and the more superficial cells. Thus the actual separation in the case of histamine does not take place between stroma and epithelium but between the actively mitotic basal layer and the more inert superficial layer. It is interesting to notice that some

properties of the two surfaces of the basal layer seem to be controlled by different mechanisms.

The effect of histamine on the cohesion of the corneal tissue differs from other pharmacological actions of histamine by the long delay.

TABLE III

Effect of Various Drugs on Adhesion of the Epithelium

Incubation Time: 20 hours.

DRUG INJECTED AMOUNT PER CORNEA	INCUBATED AT 29°C.	
	Pressure in grams	% Epithelium removed
Pontocaine		
1 mg.	40	89, 84, 85
0.5 mg.	40	78, 71, 78
0.25 mg.	40	21, 23
0.1 mg.	60	15, 12
Ephedrine		
2 mg.	40	70, 87, 73
0.5 mg.	40	15, 31
	60	52, 55
0.1 mg.	60	15, 32
Adrenaline		
1 mg.	40	92, 87, 93
0.5 mg.	40	49
	60	45
0.1 mg.	60	34, 37
Physostigmine		
1 mg.	40	80, 72
0.5 mg.	60	31, 32, 28, 36
Acetylcholine		
0.5 mg.	60	27, 35
Acetylcholine 0.5 mg. + Physostigmine 0.25 mg.	60	18, 47, 32, 27

The possibility cannot be excluded that this lag period of up to ten hours may be due to very slow penetration. Alternatively one would have to assume that the action of histamine is an indirect one, e.g. by interference with the metabolism of the epithelial cells or by af-

fecting the cells at a certain physiologic state, e.g. during mitosis. The phenomenon which is reported here differs from many other pharmacological effects of histamine also in that it is not inhibited by arginine.

Possibilities for a more prolonged action of histamine are borne out by the investigations on histamine metabolism during pregnancy (1). It is of interest to speculate as to whether the mobilization of the placenta during normal pregnancy could be caused by mechanisms similar to the epithelial loosening in the cornea produced by histamine.

SUMMARY

1. Histamine in concentrations of $5-10 \times 10^{-6}$ M. causes a loosening of the corneal epithelium. Chemically related natural bases such as histidine, tyramine, etc., have no such effect up to concentrations of 1×10^{-2} M.
2. Adrenalin, ephedrine, and physostigmine cause a loosening of the corneal epithelium in concentrations of the order of 10^{-3} M.
3. This histamine effect develops slowly after some hours of incubation. The loosening produced by histamine occurs at the boundary between the basal epithelial cell layer and the overlying epithelium, not between the basal cells and the underlying stroma as is the case with other agents which we have studied.

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IX. LOOSENING OF THE CORNEAL EPITHELIUM AFTER EXPOSURE TO MUSTARD*

HEINZ HERRMANN AND FAY H. HICKMAN

One of the prominent symptoms of mustard injury of the eye is a loosening of the corneal epithelium. This was found to occur not only *in vivo*, but also in the excised cornea incubated in a moist chamber after exposure to toxic agents. Consequently, it became possible to study the pathological process involved in the development of this symptom uncomplicated by inflammation or by reactions of the vascular and nervous systems and of other extracellular regulators. Some of the attributes of the cohesive mechanism have been analyzed by a method reported in the preceding papers. In the present experiments this method has been applied to the study of the effect of mustard on the adhesion of the corneal epithelium.

RESULTS

In the experiments to be reported here, beef eyes freshly obtained from the slaughter house were exposed to mustard vapor by resting the eye on the mouth of a cylindrical vessel 5 cm. deep, containing a thin layer of liquid mustard on the bottom. Analyses reported above (1) indicate that under these circumstances mustard enters the cornea at a rate of 0.6 micrograms per square centimeter per minute. The macroscopic appearance of the cornea changes surprisingly little after exposure of the tissue to mustard vapors for 10–20 minutes and subsequent incubation for 10–20 hours. After very severe exposures (approximately one hour) a moderate degree of corneal haze develops during incubation. With exposure of 20 minutes or more the corneas maintain their dome-like convexity better than do the controls, as if the rigidity of the stroma had increased. This phenomenon may be related to the loss of turgescence in the corneal stroma described by Cogan and Kinsey (2) after similar exposures.

The only macroscopically detectable alteration in the tissue after exposure for 15 minutes and subsequent incubation is a marked

*The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

loosening of the epithelium. The whole complex of inflammatory reactions which follow mustard exposure in tissues in vivo is here reduced to one single symptom.

1. *Exposure Time*: Using the vessels described in a preceding paragraph for the exposure, the minimum exposure time required for the production of the lesion is about 5 minutes. The full intensity

TABLE I

Adhesion of Epithelium after Exposure to Mustard Vapor for Various Periods

Separation: 2 strokes, 95 gm. pressure.

Incubation: 22 hours, 26–28°C.

CONTROL	EXPOSURE—MINUTES			
	5	10	20	60
Per cent Epithelium Removed				
12	42	95	85	13
13	19	96	75	8
7	62	86	92	2
7	25	100		6
20	38	93		9
18	37	97		20
11	76	100		8
6	84	93		7
6	39	97		6
29				8
23				7
27				8
21				
19				
21				
19				
Average... 16	47	95	84	9

of the loosening effect is observed after exposure for 10–20 minutes. However, if corneas were exposed for 60 minutes (Table I), no loosening could be observed in most experiments, and in no instance could loosening be observed when the exposure was extended to 120 minutes. An interpretation of this striking phenomenon will be attempted in the discussion later.

2. *Incubation Time*: The loosening of the epithelium develops

after a lag period. With an exposure of 10 minutes and subsequent incubation at 29–32°C., no reduction in the adhesion of the epithelium to the stroma can be detected during the first 5 hours, but the effect is fully developed after 10 hours incubation (Table II).

3. *Incubation Temperature:* When the incubation temperature was lowered, the loss of adhesion was suppressed. In the experiments in which the effect of a lowered incubation temperature was tested, the corneas were exposed to mustard for 10 minutes and then kept in a moist chamber for one half to one hour at room temperature. Following this, they were incubated at various temperatures for 20 hours and compared with controls (not exposed to mustard but incubated at the same temperature in a moist chamber). The exposed corneas

TABLE II

Adhesion of Epithelium after Varying Incubation Periods Following Exposures to Mustard Vapor

Exposure: 10 minutes.

Incubation: 26–28°C.

Separation: 2 strokes, 95 gms. pressure.

INCUBATION TIME IN HOURS AFTER EXPOSURE	PER CENT OF EPITHELIUM REMOVED					
	Control			Treated		
5	13	11	6	14	10	10
10	6	11	12	22	74	74
20	16	24		68	79	

incubated at 29°C. showed the usual decrease of the adhesion of their epithelium. Those incubated at 24°C. showed a much reduced effect. In those incubated at 11–18°C., no loss of epithelial adhesion could be observed (Table III).

As reported above, no loosening of the epithelium is observed during the first 5 hours of incubation at 29°C. The following experiment was performed in order to see how late in the symptom-free period cooling of the tissue could be instituted and still suppress the symptom. Beef corneas, after a standard exposure to mustard for 10 minutes were incubated at 29°C. for 4 to 5 hours. Thereafter they were divided into three lots. In one lot the adhesion was measured immediately and was found to be normal. A second lot was incubated at 10–18°C.

TABLE III

Per Cent Epithelium Removed after Incubation at Varying Temperatures

Exposure: 10 minutes.

Incubation Time: 20 hours.

Separation: 2 strokes, 95 gms. pressure.

CONTROL Incubation Temp. 29°C.	EXPOSED TO MUSTARD VAPOR			
	29°C.	22-24°C.	16-18°C.	11-14°C.
17	94	62	17	24
16	82	74	21	8
14	85	63	24	16
4	84	74	26	11
23	83	57		27
10	65	48		20
23	59	43		
11	66	39		
20	68	47		
17	76	43		
13	83	67		
16	88	24		
18	88	38		
8	69	39		
23	93	63		
17	86			
21	93			
20	85			
11	86			
14	82			
11	88			
77	87			
12	87			
11	87			
13	79			
	72			
	85			
	76			
	87			
	85			
Av. 15	86	52	22	18

for a further 15 hours. At the end of this period the epithelium was still normally adherent (Table IV). The third lot was incubated at 29°C. also for a further period of 15 hours. At the end of this period

this lot of corneas showed marked loosening of the epithelium. It is evident that cooling of the tissue can suppress the loss of adhesion even if cooling is applied near the end of the symptom-free period.

The effect of low temperature was tested in an additional series of experiments. Beef corneas were given a standard exposure to mustard vapor at room temperature, kept in a moist chamber at room temperature for one half to one hour, then kept for 6 to 8 hours in a cooled moist chamber (12–18°C.), and then incubated at 29°C. for 12 hours. Controls, with exposure to mustard vapor, were incubated at 29°C. also for 12 hours. We were thus able to test whether the period of cooling had any influence on the subsequent development of the lesion. Table V contains the results of all of the experiments performed of

TABLE IV

Incubation at Low Temperature Following Preliminary Incubation Period at 29°C.

Exposure: 10 minutes.

% EPITHELIUM REMOVED WITH 2 STROKES, 95 GRAMS PRESSURE		
Incubated for 4 hrs. at 29°C.	Incubated for 12 hrs. at 29°C.	Incubated for 4 hrs. at 29°C. and thereafter for 13 hrs. at 14–18°C.
18	67	10
11	87	15
26	81	17
6	85	21
	83	8
	81	

this type. While the samples that had been exposed to the preliminary cooling period gave rather widely scattered results, they show a statistically significant decrease in the degree of epithelial loosening, as may be seen by comparing the average percent epithelium removed with 95 grams weight on the scraper in the two series. The difference between these averages is 8 times the sum of their probable errors.

4. *Effect of Incubation under Anaerobiosis:* A second condition under which the loosening of the epithelium is suppressed after exposure to mustard was found to be incubation of the exposed corneas under nitrogen. For the anaerobic incubation the corneas were placed in a desiccator jar. The jar was evacuated for 15 minutes down to 50 mm. Hg, put into the incubator and then connected with a nitrogen

TABLE V
Per Cent Epithelium Removed after Incubation at 29°C., with and without Previous Incubation at 12-18°C.

Exposure: 10 minutes.

Separation: 2 strokes.

PRESSURE APPLIED, GRAMS	INCUBATION 12 HRS. AT 29°C. WITHOUT PRE- LIMINARY COOL PERIOD % EPI- THELIUM RE- MOVED	PRESSURE AP- PLIED, GRAMS	PRELIMINARY INCUBATION		SUBSEQUENT INCUBATION 12 HRS. AT 29°C. % EPITHELIUM RE- MOVED
			Temp.	Hours	
95		110	12	6	66
	83	95	12	8	62
	87				69
	66				72
	76				46
	68			6	30
					40
					57
					32
					33
	85		12	7	36
	83				26
	81	12	8	52	
	87			53	
	72	14	8	48	
	85			52	
	87			41	
	76			62	
	87			73	
	85			65	
				71	
		79.			
Av.....	80 ± 1.1				51 ± 2.4
80	87	80	18	8	69
	79				68
60		60	16	8	71
	90				71
	37				69
	12				64
	58			8	49
	63				30
	26				72
	63				28
	33				35
	49				51
	81				56
	56				37
	80				25
	58				66
	83				26
					83
					75
	Av.....				55 ± 4

tank. The gas was passed over copper wire at a temperature of about 500°C., and then bubbled through an alkaline pyrogallate solution in two separate containers, one inside, and the other outside the desiccator jar. A gentle flow of gas was maintained throughout the incubation period. In some experiments in which bicarbonate was used as an external bathing fluid for neutralization of the lactic acid which is formed anaerobically, nitrogen containing 5% CO₂ was used and the pyrogallate solution was omitted. In all of the corneas which were incubated for 10 hours under anaerobiosis after exposure to mustard for 10–20 minutes, the loosening was completely suppressed. The

TABLE VI

The Adhesion of the Corneal Epithelium under Aerobic and Anaerobic Conditions after Exposure to Mustard

Exposure: 15 minutes.

Incubation: 20 hours at 28°C.

Separation tested at a pressure of 90 gm.

CONDITIONS OF INCUBATION	NUMBER OF CORNEAS					
	Controls			Exposed to mustard		
	Epithelium					
	More than $\frac{1}{2}$ off	About $\frac{1}{2}$ off	Less than $\frac{1}{2}$ off	More than $\frac{1}{2}$ off	About $\frac{1}{2}$ off	Less than $\frac{1}{2}$ off
Aerobic.....	2	1	9	12	0	0
Anaerobic.....	0	0	13	0	0	13
10 hours anaerobic followed by 10 hours aerobic.....	1	2	13	6	4	8

same was observed with 1 ml. N/20 bicarbonate buffer as external bathing fluid which was used in order to prevent a too great drop of the pH due to the accumulation of lactic acid under anaerobic conditions.

As in the experiments with lower temperatures, we tried to obtain information as to whether the suppression of the loss of adhesion is limited to the period of anaerobic maintenance, or whether this effect is retained on readmission of oxygen. We incubated both exposed and control corneas for 10 hours anaerobically and subsequently for 10 hours aerobically, and compared these with control and exposed corneas which were incubated for 10 hours aerobically only. The complete experiment is shown in Table VI. All of the mustard

exposed corneas incubated aerobically showed loosening of the epithelium. In the series exposed to mustard and incubated first anaerobically and then aerobically, only about half showed loosening of the epithelium. This qualitative observation was supplemented by quantitative determinations of the removed epithelium which were carried out as described previously. The results of this experiment

TABLE VII
Adhesion of the Corneal Epithelium on Incubation under Aerobic and Anaerobic Conditions after Exposure to Mustard
Separation tested at a pressure of 90 gm. Removed and adherent epithelium from 3 corneas pooled in each sample.
Exposure: 15 minutes.
Incubation Temp. 29°C.

CONTROLS		% EPITHELIUM REMOVED
Incubated for 10 hours aerobically		13
Incubated for 20 hours anaerobically		13
Incubated for 20 hours aerobically		10
Incubated for 10 hours anaerobically followed by 10 hours aerobically		7
		42
		40
		42
EXPOSED		
Incubated for 10 hours aerobically		88
Incubated for 10 hours anaerobically		89
Incubated for 10 hours anaerobically followed by 10 hours aerobically		9
		9
		57
		59

agree with the qualitative findings (Table VII). None of the corneas which were kept for 20 hours anaerobically and all corneas which remained aerobic for this time showed the loss of adhesion. From these experiments it would seem that the effect of a preliminary incubation under anaerobiosis extends, in some cases, over a subsequent 10 hour period of aerobic maintenance. The results here are similar to those obtained on incubation at lower temperature. The

effect of anaerobic incubation on the epithelial loosening caused by iodoacetate, fluoride and histamine is shown in Table VIII.

TABLE VIII

Effect of Mustard and Other Agents upon the Adhesion of the Epithelium

Aerobic and Anaerobic Incubation

Separation with 90 gm. pressure.

SUBSTANCES TESTED	MODE OF APPLICATION	CONDITION OF INCUBATION	NUMBER OF CORNEAS		
			Epithelium		
			More than $\frac{1}{2}$ off	About $\frac{1}{2}$ off	Less than $\frac{1}{2}$ off
Exposure to mustard vapor for 15'	No external bathing fluid	Aerobic	34	4	0
		Anaerobic	0	0	36
	Bathing fluid 1 ml. N/20 NaHCO ₃	Aerobic	4	0	0
		Anaerobic	0	0	4
Fluoride 0.5 mg. per per cornea	External bathing fluid	Aerobic	23	0	0
		Anaerobic	0	0	22
	Injection into stroma	Aerobic	5	0	0
		Anaerobic	4	0	0
Iodoacetate 0.5 mg. per cornea	External bathing fluid	Aerobic	22	0	0
		Anaerobic	14	6	3
	Injected into stroma	Aerobic	5	0	0
		Anaerobic	4	0	0
Pontacaine 0.5 mg. per cornea	External bathing fluid	Aerobic	4	0	0
		Anaerobic	0	3	1
	Injected into stroma	Aerobic	4	0	0
		Anaerobic	4	0	0
Histamine 20 micro-gms. per cornea	External bathing fluid	Aerobic	1	0	3
		Anaerobic	0	0	4
	Injected into stroma	Aerobic	4	0	0
		Anaerobic	0	0	4

DISCUSSION

Loss of cohesion between the corneal epithelium and stroma is a prominent feature of mustard injury. In vivo this symptom is

commonly associated with vascular and cellular inflammatory reactions. The experiments reported in this paper show that the loss of cohesion is not a consequence of inflammation and may be studied *in vitro* in the absence of any inflammatory reaction. Moreover, the loss of cohesion is not a manifestation of death of the epithelial cells. The experiments reported in one of the preceding papers (3) show that, even when the epithelium is loosened, the epithelial cells still maintain their capacity to respond by migrating in a completely normal fashion in the healing of small wounds. It is true that under ordinary experimental conditions, scattered cells in the basal layer show nuclear fragmentation and are evidently dying at the time that the loosening of the whole epithelial layer takes place. Nuclear fragmentation, however, has a larger temperature coefficient than epithelial loosening, and it is possible, by incubating at 25–30°C., to elicit the phenomenon of epithelial loosening without, or, at any rate, long before the appearance of nuclear fragmentation. Further evidence that the loss of cohesion is not a manifestation of tissue death is seen in the experiments which show that at lowered temperature or under anoxia the tissue is able to some degree to recover from the injury that otherwise leads to epithelial loosening.

Loss of cohesion between corneal epithelium and stroma appears to be an example of a rather widespread type of reaction to mustard injury. With adequate dosage the corneal endothelium sloughs off, and the loss of this cellular boundary leads to a severe oedema of the corneal stroma. Histologic study reveals that the capillary endothelium in the conjunctiva is similarly affected, and that after exposure to mustard many capillaries are converted into tubes devoid of endothelial lining. In many of these lesions petechial hemorrhages occur (4). In others the leakage of plasma through these denuded channels results in a close packing of the red cells and finally in a stoppage of circulation in the affected vessels. Similar changes are observable in the iris vessels after exposure to the more penetrating of the nitrogen mustards. To what extent this circulatory failure contributes to the development of tissue necrosis in severe lesions is still unclear, but it is certainly not without a significant effect on the whole pathologic process. Loss of cohesion between the epithelial cells has been shown by Danielli (5) to be an important factor in the production of vesication by mustard in the frog's skin, quite independent of the level of

extra-vascular tissue fluid pressure. To what extent all these pathologic processes are to be attributed to the same or similar mechanisms remains obscure. As noted in a preceding paper, the frog's skin and the beef eye behave differently in respect to loss of cohesion following exposure to certain metabolic poisons. Moreover, in the vesication in the skin produced by mustard, the loss of cohesion occurs between layers of epithelial cells, not primarily between epithelium and connective tissue as in the cornea.

In the development of all of the symptoms of mustard injury that have so far been studied, three phases are recognizable. There is first the primary chemical reaction of mustard with tissue components. In the cornea this is practically completed within a few minutes. There follows a symptom-free period during which neither physiological nor histopathological changes are recognizable. Poisoned nerves continue to conduct, muscles to contract, glands to secrete. The duration of the symptom-free period varies with the dose and with the symptom studied. In respect to epithelial loosening in the cornea the lag period is 6-12 hours.

If we speak of the mechanisms of mustard injury we assume that between the primary chemical reaction of mustard with tissue and the subsequent development of recognizable symptoms there exists a determinate series of events, but we are still almost completely in the dark as to that series of events. The present study has shown only that oxygen is required for the development of a particular symptom, but whether the symptom is due to the opening of some new oxidative channel in the cellular respiration, or to the inhibition of some normally compensating reductive process, remains obscure. Failure of the symptoms to develop after exposure to excessively large doses of mustard suggests that under these conditions the oxidative pathway, or some other system necessary for the development of this symptom, is inhibited.

The ability of fluoride and iodoacetate to produce epithelial loosening under anaerobic conditions indicates that these agents injure the normal mechanisms for the maintenance of tissue cohesion at some points other than, or in addition to, the injury produced by mustard. Since these agents are known to inhibit glycolysis, a first assumption would be that the glycolytic mechanism is involved in the maintenance of normal cohesion, but fluoride and iodoacetate, like mustard, damage

many unrelated intracellular mechanisms and, until more positive evidence is available, no specific conclusions can be drawn regarding the locus of their effect.

This analysis exhibits the pathological mechanisms of mustard injury as a process perhaps comparable in its complexity to that of normal physiological mechanisms, and suggests that the further study of this pathological mechanism may be useful in throwing light on the related physiological mechanisms. The obvious approach to such a study lies in the biochemical investigation of the tissue following mustard injury, and it is to this field that the succeeding papers in this series are devoted.

SUMMARY

1. Loosening of the corneal epithelium following exposure to mustard occurs both in vivo and in the isolated tissue incubated supravivally. It is independent of any inflammatory reaction.
2. The symptom develops after a period of incubation of 6-12 hours following exposure.
3. Lowering of the temperature or incubation in the absence of oxygen not only suppresses the symptom during the period of application of these conditions, but enables the tissue to recover partially from the injurious effect.
4. It is concluded that the symptom results either from some abnormal utilization of oxidative energy or from the inhibition of some normally balancing reductive process.

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X. EXPLORATORY STUDIES ON CORNEAL METABOLISM*

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The previously available knowledge on corneal metabolism is extremely limited, and has been reviewed in an earlier paper (1) from this laboratory. The present study was undertaken in order to establish some background on the basis of which metabolic effects of mustard injury could be sought for. These studies are in no sense complete. Several of the more interesting metabolic pathways discovered were not investigated further when it was found that they were unaffected by exposure of the tissue to mustard. Nevertheless, a general picture of the chief pathways of energy metabolism in the cornea has emerged.

For an investigation of the tissue metabolism the cornea offers the following advantages. It contains an endogenous store of reserve metabolites sufficient to maintain respiration and fermentation at the initial level in the excised tissue for at least one day. On the other hand, it is easily possible to supply to the cells of the excised tissue metabolites or inhibitors, either by irrigation, or by injection into the stroma. The latter procedure is of importance for the supply of relatively high concentrations of substances which are available in only small amounts. Because of its unique histological properties, metabolic rates can be measured without slicing or macerating the intact tissue. Furthermore, it is possible to separate the tissue components mechanically in order to use homogeneous samples of cells of special types, and to investigate possible metabolic interactions between tissue components. Experiments with corneas thus combine in many respects the advantages of metabolic studies on tissue slices and on whole organs, while at the same time furnishing almost uniquely simple opportunities for the study of intercellular metabolic processes.

METHODS

The experiments were performed on beef corneas. The preparation and supravital maintenance of the corneas have been described be-

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

fore (2). Determinations of extractable carbohydrates, lactic acid, and phosphates were carried out on trichloroacetic acid extracts. Three corneas were pooled in each sample and were suspended in a total volume of 10 ml. of 5% trichloroacetic acid. A satisfactory extraction requires vigorous and thorough grinding for 10 to 15 minutes.¹ Insoluble portions of the tissue were separated either by centrifugation, or by filtration through Whatmann Filter paper No. 42, (if phosphorus determinations are to be carried out).

Extractable carbohydrates were determined by Hoffmann's method (3); 0.2 to 0.5 ml. of the filtrates were used. The excess of acid was neutralized by addition of N/1 NaOH (yellow to thymolblue) and the volume was made up to 1 ml. with distilled water. 2 ml. of the ferricyanide reagent were added and the determination continued as described in the original procedure. This method is not very specific but it was satisfactory for our purpose, since in most experiments merely the rate of disappearance of an excess of injected glucose was measured.

Lactic acid was determined according to the method of Barker and Summerson (4).

Inorganic phosphorus was determined according to the method of Lohman and Jendrassik (5), adapted for use with photoelectric colorimeter (Klett Summerson Filter 660). The organic phosphate fractions were determined by differential hydrolysis, as described by Lohman (6). The hydrolysis was carried out with N/1 HCl in a boiling water bath. Three fractions were obtained, the first two after hydrolysis for 7 to 180 minutes respectively, and the third after digestion with concentrated perchloric acid for $\frac{1}{2}$ hour. We found it to be convenient to hydrolyze the samples for each particular time individually. For this purpose aliquots of 0.5 ml. of the filtrate were pipetted into narrow 5 ml. serological tubes, and 0.5 ml. of 2N HCl was added. The tubes were closed with tightly fitting vaccine bottle stoppers and immersed in a boiling water bath. After the required time, the tubes were removed and cooled. The contents were transferred into Klett tubes, 1 ml. of the molybdate reagent and 0.2 ml. of the amino-naphthol-sulfonic acid reagent were added and the volume

¹ A mechanical device which can be used as a substitute for hand grinding in preparing corneal extracts is described in Appendix II.

made up to 5 ml. The samples which were digested with concentrated perchloric acid were also transferred to Klett tubes and the pH was adjusted to between 3 and 8 (yellow to thymolblue) by addition of about 0.8 ml. of 40% NaOH. Double amounts of reagents were added and the volume was finally made up to 10 ml. The color in all samples was developed in five minutes at 37°C.

Glycogen was estimated according to Sjögren's (7) modification of Pflüger's method. We found the determination to be practicable only when applied to the epithelium previously mechanically separated from the stroma. In the presence of stroma, extraction with alkali, the first step in the analytic procedure, yields large amounts of corneal mucoid in the extract, and attempts to precipitate glycogen by addition of alcohol to this mixture were unsuccessful. Epithelium from three corneas was digested in 0.5 ml. of 30% NaOH in boiling water bath for 30 minutes. The volume was brought to 1 ml. and 1.2 ml. of 95% alcohol were added. After 3 re-precipitations and hydrolysis in N/1 HCl for 3 hours, the amount of glycogen was determined as glucose with Hoffmann's method. In all colorimetric procedures standard samples were carried along for each set of determinations.

Oxygen uptake was measured in the Warburg apparatus with one cornea per flask bathed in 3 ml. M/20 phosphate buffer of pH 7.2-7.5. Anaerobic glycolysis was measured similarly using 3 ml. Ringer's bicarbonate solution for the bathing fluid in an atmosphere of 95% N₂ with 5% CO₂. The sodium hexosediphosphate used for the injection in some experiments was prepared from the commercially available calcium salt (Schwartz Lab) by precipitation of the calcium with an equivalent amount of sodium oxalate. All other reagents were the purest commercial samples. All solutions for injection were adjusted to pH 7.

In the case of the cornea, the use of the dry weight unit is misleading as the standard of reference for the calculation of metabolic rates. The inadequacy of such calculations for heterogenous tissues in general has been discussed by Berenblum et al. (8). These authors propose to relate the metabolic activity to the number of cells and to use the nucleic acid phosphorus as its chemical index. However, there does not seem to be any reason to assume that the nucleic acid content is an adequate index of the cell number since the nucleic acid content per

cell may vary greatly from tissue to tissue. In the cornea we had an opportunity to determine cell number and nucleic acid content in the stroma and in the epithelium, and to check in this way the assumed correlation. Microtome sections of the cornea cut vertically to the surface and of 8 micra in thickness were prepared. Cell counts were carried out in an area of $\frac{1}{2}$ mm. width. In the given area we obtained for the epithelium counts of 102, 112, 87, 114 cells, and for the stroma 56, 50, 56, 48.² The number of cells in the endothelium is about one-tenth that in the epithelium. As was pointed out in a previous paper (9), not all of the stroma cells are keratocytes. In the beef cornea 3-5% of the stroma cells are wandering cells.

The determinations of nucleic acid were carried out on the separated epithelium and stroma from several corneas according to the procedure described by Berenblum. We found about 40 micrograms of nucleic acid phosphorus in the epithelium and 10 micrograms in the stroma.³ There is no correlation between these two sets of figures, and the nucleic acid content is apparently only a very crude index of the number of cells.

Since our metabolic studies have been carried out on the whole cornea and were aimed at a metabolic interpretation of the functional state of the histological units of the whole tissue, we considered the expression of the metabolic activity per cornea or per cell as the most adequate. Pooling 3 corneas for each determination and extending the number of the experiments yielded relatively stable mean values. In order to render the various systems of reference convertible, we have compiled all pertinent data, derived from our own determinations and from Krause's "Biochemistry of the Eye", in Table I.

EXPERIMENTAL RESULTS

(1) *Oxygen consumption.* Some data on the respiration of the cornea are available in the previous literature (1). For the purposes of the present study we have extended the previous results to include the O₂ uptake of the tissue at 32°C., since this was the temperature of incubation found most convenient for the study of the carbohydrate

² We are indebted to Dr. Buschke and Dr. Friedenwald for most of the histologic data.

³ The value for the stroma is probably too high due to probable unavoidable contamination from the large amount of collagen in the tissue.

metabolism. At this temperature the oxygen uptake of the whole cornea was found to average 70 cu. mm. per hour. In corneas that had been denuded of their epithelium, the oxygen uptake was reduced to about 10%. If the endothelium is also removed less than 3% of the original oxygen consumption remains, and if this figure is corrected for the auto-oxidation found in boiled corneal stroma less than 2% of the respiratory uptake of the cornea remains to be attributed to the stroma, though the tissue contains approximately one-third of all the corneal cells. It must be remembered, moreover, that 3-5% of the stroma cells are wandering cells, and evidence has been presented in a previous paper (9) indicating that these wandering cells probably have

TABLE I
Reference Data on Beef Cornea
Whole Cornea

Greater Diameter.....	25- 30 mm.		
Lesser Diameter.....	16- 21 mm.		
Wet Weight.....	480-630 mg.		
Dry Weight.....	90-120 mg. (19% of wet weight)		
	EPITHELIUM	STROMA	ENDOTHELIUM
Wet weight.....	70-90 mg.	400-600 mg.	7-8 mg.
Dry weight.....	16-18 mg.	80-110 mg.	
Thickness.....	about 0.1 mm.	about 0.7 mm.	about 0.005 mm.
Number of cells.....	$4-10 \times 10^7$	$2-5 \times 10^7$	$1-2 \times 10^6$
Nucleic acid phosphorus....	40 micrograms	less than 10 micrograms	

an aerobic metabolism since their pathological responses differ under aerobic and anaerobic incubation. If the oxygen consumption per wandering cell is equal to that per cell in the epithelium or endothelium, the total oxygen uptake of the stroma would be entirely accounted for. This leads to the conclusion that the keratocytes are probably completely devoid of respiratory oxygen uptake.

(2) *Glycogen*. The epithelium of 50 corneas was worked up according to Sjögren's method. After fivefold reprecipitation with alcohol, about 50 mg. of a white powder was obtained. A comparison of this substance with commercial glycogen (Schwartz' Lab) showed very similar rates of liberation of reducing groups during hydrolysis with HCl or with diastase, and an almost identical decrease in specific

rotation (Fig. 1). From the acid hydrolysates of both samples, phenylosazones were prepared which showed the characteristic crystal form of glucosazone and had a melting point of 203–204°C., which remained constant after mixing the two substances.

The corneal epithelium contains 1 to 1.5 mg. glycogen per cornea. In the isolated surviving cornea this carbohydrate reservoir is slowly depleted. The rate of depletion varies with temperature, being almost zero at 24°C. and about 25 micrograms per cornea per hour at 32°C.

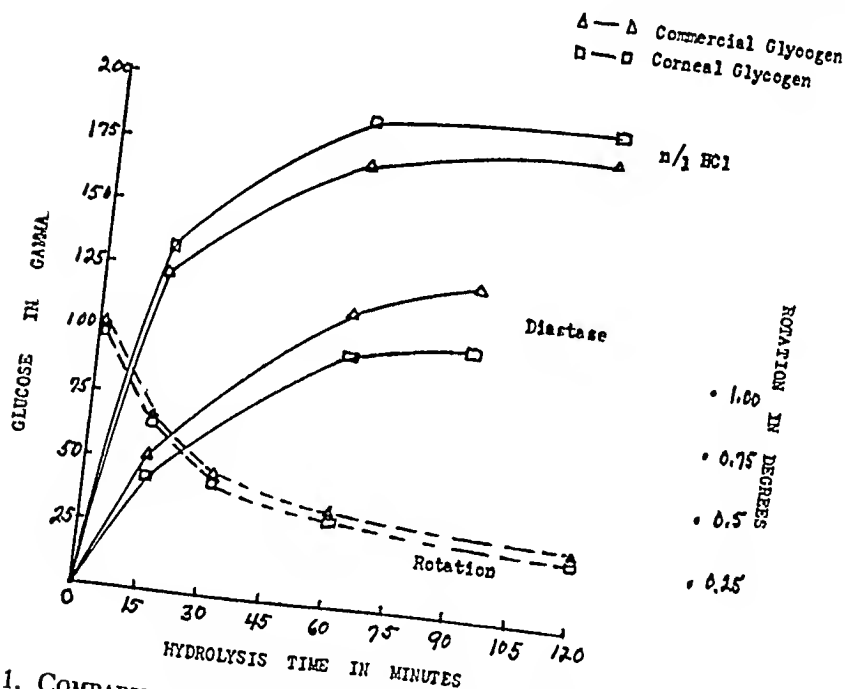


FIG. 1. COMPARISON OF HYDROLYSIS RATES OF COMMERCIAL AND CORNEAL GLYCOPEN.

(Table II). The rate of disappearance was found to be undisturbed by iodoacetate. After the injection of glucose the rate of disappearance of glycogen was diminished. No attempt was made to decide whether this effect was due to an inhibition of the breakdown of glycogen or to an accelerated resynthesis. However, even when as much as 10 mg. of glucose was injected into corneas no increase in the glycogen level was observed.

(3) *Glucose utilization and lactate content.* In vivo, the epithelium receives a continuous carbohydrate supply, in the form of glucose, from the tears (60 mg. % glucose) and through the stroma from the

TABLE II

Glycogen Content of Epithelium of Excised Corneas after Various Incubation Periods: Effects of Iodoacetic Acid and Glucose

Incubation at 32°C.

Micrograms Glycogen per Cornea

INJECTED SUBSTANCES MG. PER CORNEA	BEFORE INCUBATION	AFTER INCUBATION			
		10 hours		20 hours	
		Control	Injected	Control	Injected
No injection	908	848		600	
	1066	773		625	
	1100	833		575	
	1543				
	1088				
Iodoacetate, 0.5 mg.		866	966	608	516
		760	700	550	575
		785	991	566	508
		816	925	725	734
Glucose, 2 mg.	1175	640	1000	628	1050
		623	1025	391	1283
		833	1190		
		816	916		
		908	1250		
		941	1083		
Glucose, 10 mg.			1173		
			1106		

Average Glycogen content of fresh tissue, 1147 micrograms.

Average Glycogen content after incubation in presence of glucose, 1107 micrograms.

Glycogen consumption in the absence of injected glucose:

First 10 hours of incubation 34 micrograms per cornea per hour

Second 10 hours of incubation 22 micrograms per cornea per hour

capillaries in the limbus, and perhaps from the aqueous. We tried to imitate the natural conditions by irrigation of the cornea. The effects of two irrigation fluids, Ringer's solution and Ringer's plus glucose (0.1%) were compared.

The excised corneas were placed on glass slides (5 x 7.5 cm.) which were fastened to an inclined piece of sheet metal. Each cornea received the irrigation fluid from a separate 500 ml. Erlenmeyer flask through glass tubing and rubber connections, the number of drops being regulated by a screw clamp to a rate of about one drop every ten

TABLE III
Oxygen Uptake, Anaerobic Acid Production, and Lactic Acid Content of Beef Corneas After 23 Hours of Irrigation

Anaerobic Acid production measured as CO₂ output. 100 cu. mm. CO₂ is equivalent to approximately 400 micrograms of lactic acid.
The Lactic Acid content of "Controls" was measured after 23 hours irrigation without subsequent manometric measurements.

IRRIGATING FLUID	O ₂ UPTAKE IN CU.MM. PER HOUR PER CORNEA	CO ₂ OUTPUT IN CU.MM. PER HOUR PER CORNEA	LACTIC ACID CONTENT MICROGRAMS PER CORNEA
Ringer	70	95	48
	62		58
Ringer + 1% Glucose	62 68 61 58	121 105	470 } After 1 hr.
			560 } Anaerobiosis
			62 Control
			295
			630
Ringer + Glucose + M/1000 Iodoacetate	42	22 27	510
			220
			1270 } After 1 hr.
			860 } Anaerobiosis
			335 Control
			63
			13 } After 1 hr.
			71 } Anaerobiosis
			0 Control

seconds. Air pressure from a central regulator kept the dropping rate constant for several hours.

The corneas were maintained under irrigation for 20 hours at room temperature. Then the lactic acid content and the rate of respiration and of anaerobic glycolysis were determined. The two latter determinations were carried out in Warburg manometers with 2 half corneas per flask at a temperature of 38°C. (Table III). The rate of both O₂

uptake and anaerobic acid production after the irrigation period were found to be at the same level as in the fresh cornea. The addition of glucose has no apparent stimulating effect on the respiration, and even the anaerobic glycolysis is the same after the irrigation with Ringer's solution and with Ringer's plus glucose.

A great difference between the two irrigation fluids was observed in their effects on the lactate level of the irrigated corneas. A considerably higher lactate content was found in the corneas irrigated with the Ringer's solution which contained glucose. This effect of glucose is abolished if iodoacetate in a concentration of M/1000 is added to the irrigation fluid. In this case, the respiration was found to be inhibited by about 30% and the anaerobic glycolysis by 80%. Discrepancies of this type between the inhibitions by iodoacetate of oxygen consumption and of anaerobic acid production have been the subject of wide discussion (Barker, Shorr, and Malam (10)).

Extracts can be obtained from fresh excised corneas which reduce Hoffmann's Ferricyanide reagent. Some reduction takes place at room temperature and titrations with dichlorophenol indophenol indicate that about $\frac{1}{3}$ to $\frac{1}{2}$ of the total reduction values at high temperature are due to ascorbic acid. Pirie (11) has recently reported that almost all of the ascorbic acid in the cornea is in the epithelium, and our own experiments are in accord with her results. Calculated as glucose the total reducing values correspond to about 200-300 micrograms per cornea. Corrected for ascorbic acid this would correspond to 100 to 200 micrograms glucose per cornea. The total reduction value decreases during the incubation, and this change apparently concerns only the non-ascorbic acid fraction. However, no extensive experiments were carried out on the state of the endogenous reducing material. For a more quantitative study of glucose utilization in the excised cornea, we injected glucose into the stroma and observed its disappearance. We used amounts of glucose which were great compared with the quantity of endogenous reducing material. In that way changes in the concentration of the latter during the incubation could be neglected.

We found that the injected glucose is rapidly utilized on incubation, about 100 micrograms disappearing per hour, per cornea (Table IV). Experiments with the separated stroma showed that considerable

TABLE IV
Glucose and Lactic Acid Content of Beef Cornea. Effect of Injected Glucose, Iodoacetate, and Fluoride
 Incubation 10-12 hours, at 32°C.

		INJECTED PER CORNEA	GLUCOSE MICROGRAMS PER CORNEA		LACTIC ACID MICROGRAMS PER CORNEA	
			Before incubation	After incubation	Before incubation	After incubation
Whole Cornea	No injection	319	115		130	
		352	116		135	
		232	176		95	
		165	117		90	
	Glucose 2 mg.	1650	701		620	
		1618	306		600	
1392		347		750		
1419		351		620		
Denuded Stroma	Glucose 2 mg.	1660	1200	300 350	605	
		1780	1120		565	
			(20 hours incubation)		(20 hours incubation)	
			840		550	
			860		510	
					505	
Whole Cornea	Iodoacetate 0.5 mg.		306		300	
			264		255	
	Iodoacetate 0.5 mg. Glucose 2 mg.		1285		285	
			1171		255	
	Glucose 0.5 mg.		160		203	
			166		110	
			200		223	
			210		150	
	Glucose 0.5 mg. Iodoacetate 0.5 mg.		543		143	
			500		56	
			580		40	
			590		90	
	Glucose 0.5 mg. Fluoride 1.0 mg.		323		370	
			400		436	
			320		400	
			355		440	
Glucose 0.5 mg. Fluoride 1.0 mg. Iodoacetate 0.5 mg.		516		123		
		586		110		
				105		

amounts of glucose are utilized in this tissue component. With only $\frac{1}{2}$ of the cell number of the epithelium, the rate of utilization of glucose per cell seems to be about twice as high in the isolated stroma as in the epithelium.⁴ The lactate content remains at the original level or increases slightly during incubation if as much as 2 mg. of glucose are injected per cornea. Smaller amounts of glucose (0.5 mg.) are not sufficient to maintain the lactate content at the initial level but retard its disappearance. Addition of iodoacetate inhibits the disappearance of glucose. 0.1 mg. of iodoacetate per cornea (about M/1000) still exhibits the full effect. The rate of disappearance of lactate in the presence of iodoacetate and glucose is the same as with iodoacetate alone and it is only slightly less rapid than in the untreated controls.

The inhibition of glucose disappearance by fluoride (about M/50) is less complete than that exhibited by iodoacetate, but the decrease of the lactate level is considerably retarded as compared with the effect of iodoacetate. This retardation is observed also in the absence of glucose. Iodoacetate applied in addition to fluoride increases the inhibition of the glucose disappearance and accelerates the disappearance of lactate. Thus it supersedes in both respects the action of fluoride. The retardation of the lactate disappearance both by iodoacetate and by fluoride will be considered in greater detail below.

(4) *Phosphate fractions.* The gradual depletion of the glycogen stores in the cornea during incubation, as well as the inhibiting effect of iodoacetate upon the rapid utilization of glucose, suggested the presence of active phosphorylating mechanisms, and led us to the examination of free and esterified phosphate in the cornea under various experimental conditions.

We determined 4 phosphate fractions. The phosphate which is determined directly without hydrolysis (IP) is equal to the sum of inorganic phosphate plus creatine phosphate, plus acetyl phosphate. The phosphate (ATP) liberated within the first 7 minutes of hydrolysis with HCl originates mainly from adenosine triphosphate, and the phosphate liberated during further hydrolysis up to 180 minutes (DP) is derived essentially from hexose diphosphate. The excess of phosphate found after digestion with perchloric acid is derived from such esters as phosphoglycerate (PG).

⁴ More detailed studies in regard to glucose utilization are to be found in a succeeding paper (12).

The content of these fractions was determined in the whole cornea and in the epithelium and stroma separately, both in fresh corneas and after an incubation for 20 hours at 32°C. (Table V). One would have expected that the figures for the whole cornea should equal the

TABLE V
Extractable Phosphate Fractions
Micrograms Phosphorus per Cornea

	IP			ATP			DP			PG		
	Whole cornea	Epithelium	Stroma	Whole cornea	Epithelium	Stroma	Whole cornea	Epithelium	Stroma	Whole cornea	Epithelium	Stroma
Fresh tissue	35	32	22	16	8	3	28	28	2	31	24	18
	42	27	28	15	13	7	27	26	3	33	36	14
	24	28	21	26	18	2	18	14	7	30	39	18
	25	25	24	20	7	3	19	22	5	32	38	16
	26	24	23	19	11	4	29	27	3	42	33	23
	31	26	22	18	9	5	25	22	5	41	34	19
	34	27	21	23	8	3	33	29	7	35	24	25
	35	28	23	22	10	5	30	28	7	38	23	25
Average.....	31	27	23	20	11	4	26	25	5	35	31	20
After 20 hours, Incubation at 32°C.	18	16	18	17	14	3	19	14	5	34	30	17
	22	19	19	14	12	4	24	16	7	32	31	28
	26	18	19	22	9	1	18	27	8	22	30	22
	25	16	24	11	13	2	34	23	12	34	23	25
	21	16	20	18	23	4	22	21	9	31	32	23
Average.....	22	17	20	16	14	3	23	20	8	31	29	23

IP = Phosphate determined directly i.e. inorganic phosphate + creatine phosphate + acetyl phosphate.

ATP = Phosphate determined after 7 minutes hydrolysis with N/1 HCl minus IP, i.e. chiefly adenosine triphosphate.

DP = Phosphate determined after 180 minutes hydrolysis with N/1 HCl minus (IP + ATP) i.e. chiefly hexose diphosphate.

PG = Phosphate determined after digestion with perchloric acid minus (IP + ATP + DP) i.e. chiefly glycerophosphate.

sum of the values obtained for the separated epithelium and stroma. A good correspondence was observed with the fractions ATP and DP, of which a considerably higher concentration is found in the epithelium. These two fractions are bound to the cells, but even if calculated per

cell the concentration in the epithelium of ATP and DP are higher by 30% and 50% than in the cells of the stroma. In the IP and PG fractions, of the separated tissue components, we obtained values which were twice as high as the values for the whole cornea. Since the increase is not accounted for by a corresponding decrease in the other extractable fractions, a liberation of extractable phosphate from inextractable precursors has to be assumed. It is known that by slight damage various enzymatic reactions are started in the cell, e.g. aerobic glycolysis, and it is not impossible that the trauma of the separation activates enzymes which split IP and esters of the PG fraction from such inextractable precursors as nucleic acids, phospholipids, or phosphoproteins (13). This makes it impossible to give valid data on the distribution of PG and IP fractions between stroma and epithelium in the whole intact cornea. However, the epithelial cells are more exposed to injury in the course of the separation, the stroma cells being protected by the collagen sheets; consequently liberation of the extra P probably occurs in the epithelium. If this assumption is correct that most of the excess P is produced in the epithelium, the stroma would normally contain a much larger amount of these two fractions per cell than the epithelium. This seems probable since IP and PG are not necessarily bound to the cells and the excess could be due simply to the greater bulk of the stroma.

Values for fresh tissues change little on incubation. That this stationary state is not merely the result of a metabolic sluggishness of the cornea can be seen from the results in Table VI. They show that the levels of the various phosphate fractions in the cornea are sensitive to the influence of substrates and inhibitors. Marked alterations occur after injection of iodoacetate. The most prominent change takes place in the DP fraction, particularly when phosphate is supplied in addition to iodoacetate. Under these conditions the fraction increases by 40-50 micrograms per cornea. At the same time the IP fraction decreases by about 30 micrograms and the ATP fraction by 5-10 micrograms. Thus, practically the entire increase of the DP fraction is accounted for.

(5) *Lactate utilization.* In the preceding section, we showed that in vivo lactate is formed in the cornea by the continuous supply of glucose. For this reason an appreciable lactate content can be found in the freshly excised cornea which amounts to about 350 micrograms per

cornea; between one fourth and one fifth of this lactate content can be found in the epithelium, which forms only about one seventh of the total volume of the cornea. The concentration in the epithelium is, there-

TABLE VI
Phosphate Fractions in Whole Beef Cornea Effect of Iodoacetate
The figures represent the average values of 4-8 samples each.

INJECTED PER CORNEA		PHOSPHORUS IN MICROGRAMS PER CORNEA			
		IP	ATP	DP	PG
		91			
Sodium Phosphate 0.5 mg.	Examined before incubation				
No injection	Examined after 10 hours incubation at 32°C.	22	17	27	28
Iodoacetate 0.5 mg.		14	8	44	28
Iodoacetate 0.5 mg. + Phosphate 0.5 mg.		64	9	77	22

TABLE VII
Lactic Acid Content of Corneas after Varying Periods of Incubation at 32°C.

LACTIC ACID CONTENT IN MICROGRAMS PER CORNEA			
Incubation Time			
0 hours	4 hours	8 hours	11-12 hours
330	250	150	10
310	230	110	10
320		170	40
390		200	70
420		190	40
400		160	20
		170	40
			10
Av.....360	240	160	30

fore, somewhat higher than in the stroma. Without glucose supply, the lactate depot is gradually depleted at a rate of about 25 micrograms per hour per cornea, and after about 12 hours it has almost completely disappeared (Table VII). If, however, the epithelium is

first separated from the stroma and the two tissue components are incubated separately, then the lactate disappearance in the stroma, which contains about three fourths of the total amount present in the cornea, is practically eliminated and the lactate level in the separated

TABLE VIII

Lactic Acid Content Determined in Stroma and Epithelium Separately

Iodoacetate: 0.5 mg. per cornea.

		LACTIC ACID CONTENT IN MICROGRAMS PER CORNEA		
		Incubation Time in Hours		
		0	8	22
Stroma and Epithelium separated prior to incubation	Epithelium		250	266
	Epithelium + Iodoacetate		300	327
	Stroma		90 30	209 126
	Stroma + Iodoacetate		318 259 375 275	
Stroma and Epithelium separated after incubation	Epithelium		260 300	
	Epithelium	84 68 80	36 59 45	27 18
	Stroma	324 316 301	153 42 210 243	0 5
	Stroma			

epithelium seems to increase (Table VIII). However, if iodoacetate is added, the normal lactate disappearance is found in the separated epithelium while no effect of iodoacetate can be observed on the inhibited lactate disappearance in the stroma. This could mean that in the case of the epithelium, the increase is due to the aerobic glycolysis which ensues after cell injury. Such a process is unlikely in the case

of the stroma because here the cells are protected from injury by thick covers of collagen.

The dependence of the lactate level in the whole cornea upon glucose and iodoacetate is demonstrated in a greater number of experiments

TABLE IX
Effect of Varying Iodoacetate Concentrations upon the Lactic Acid Content of Corneas
Incubation Time: 10 hours.

IDOACETATE INJECTED PER CORNEA	LACTIC ACID CONTENT IN MICROGRAMS PER CORNEA
0	100, 105
0.25 mg.	120, 150
0.5 mg.	190
1.0 mg.	170

TABLE X
Lactic Acid Content in Corneas after the Injection of Glucose, Iodoacetate, and Glucose + Iodoacetate
Incubation: 12 hours at 32°C.

MICROGRAMS LACTIC ACID PER CORNEA						
Control	Iodoacetate .5 mg. per cornea	Glucose mg. injected per cornea				Glucose (2 mg. per cornea) + Iodoacetate (.5 mg. per cornea)
		6.0	2.0	1.0	0.6	
50 130	180 160	470	420	330	180	170
90 40	70 250	450	210	440	220	220
80 110	150 240	530	390	310	170	200
30 110	150 80		500	430		190
20 170	130 150		590	340		180
90 40	90		37	330		240
80 80	80		42			200
100 40	110		180			250
60 130	170					
60	180					
Av.....80	146	480	385	363	180	206

listed in Tables VIII to X. In the whole cornea iodoacetate itself inhibits the lactate disappearance only slightly (Table IX), but it restores the lactate disappearance after its suppression by glucose (Table X). Glucosamine shows a similar effect to that of glucose

TABLE XI
Effect of Injection of Glucosamine,

Incubation: 12 hours at 32°C.

Control	Iodoacetate .5 mg. per cornea	Glucosamine mg. injected per cornea			Glucosamine (2 mg. per cornea) + Iodo- acetate (0.5 mg. per cornea)
		6.0	2.0	0.6	
					300
	180	160	260	240	100
50	70	230	330	190	190
90	150	290	250	260	240
80	130	130	280	200	150
30	130	270	370	210	190
20	240	290	300	350	300
80	80	170	350	220	270
90		180	420	220	110
60		170			230
130		300			200
40					
40					
80					
40					
Av...64	150	230	320	236	207

Av...64	150	230	320	400
---------	-----	-----	-----	-----

tions. Under the experimental conditions used by this author, neither lactate nor pyruvate could be found among the breakdown products. The elucidation of the metabolism of glucosamine in the cornea is desirable, since this amino sugar is a constituent of the mucopolysaccharides which are of importance for the maintenance of the transparency of the cornea. Among a larger series of potential lactate precursors, other than glucose and glucosamine, only hexosediphos-

TABLE XII

Lactic Acid Content of the Cornea after Injection of Various Metabolites
Incubation: 10 hours at 32°C.

INJECTED SUBSTANCE PER CORNEA	MICROGRAMS LACTIC ACID PER CORNEA	
	Control	Injected
Hexose diphosphate, 2 mg.	76	233
	60	220
	73	243
Ascorbic Acid, 2 mg.	73	50
	60	60
dl Alanine, 2 mg.	47	138
	90	170
Glycine, 2 mg.	90	75
Glutamic Acid, 2 mg.	90	96
		90
Phosphate, 0.5 mg.	93	100
	121	108
Succinate, 0.5 mg.	80	60
	80	70
Fumarate, 0.5 mg.	130	110
		140
		140
Malonate, 0.5 mg.		110
		130

TABLE XIII

Effect of Fluoride on Lactic Acid Content of Cornea

Incubation: 10 hours at 32°C.

FLUORIDE INJECTED PER CORNEA	MICROGRAMS LACTIC ACID PER CORNEA
None	85, 57, 99, 95 60, 40, 123, 130
0.01 mg.	260
0.03 mg.	320, 258, 242, 145
0.1 mg.	432
1.0 mg.	326, 263, 316, 378, 425
Iodoacetate 0.5 mg.	100, 73, 100, 131, 83
Iodoacetate + 1 mg. Fluoride	176, 150, 156, 111, 178

phate had a qualitatively similar effect, although quantitatively less intense, upon the lactate disappearance (Table XII).

Other conditions, under which the inhibition of lactate disappearance is removed by iodoacetate, are injection of fluoride (Table XIII) and lower temperature (Table XIV). An explanation of this apparently paradoxical effect will be attempted in the following discussion.

TABLE XIV

Lactic Acid Content of Corneas after Incubation for 12 Hours at Room Temperature with and without Injection of Iodoacetate

Injected: 0.5 mg. Iodoacetate per cornea.

MICROGRAMS LACTIC ACID PER CORNEA	
Controls	Iodoacetate
240	146
223	170
256	156
245	141
Av.....241	153

DISCUSSION

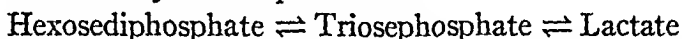
In the experimental part of this paper, several compounds have been demonstrated in the corneal epithelium, which are expected to be present in a tissue with an active glycogenolysis or glycolysis. The glycogen content of the epithelium and of muscle are nearly equal (1%) and the phosphate fractions are found to be of the same order of magnitude as in the intestinal mucosa (15). A compilation of the adenosine polyphosphate level of various organs (16) shows that the epithelium of the cornea contains more of this fraction than liver, kidney, and brain and approximately as much as muscle. The lactic acid content is about 5-7 times higher in the epithelium (200 mg. %) than it is in the blood under conditions of rest and it also exceeds the content of the blood after severe exercise (17).

Most of these figures represent steady states, the level of which depends upon the rate of formation and consumption of the substance under observation. A shift of these levels will become evident if either formation or consumption are interfered with; simultaneous inhibition

or acceleration of both will lead to a change in the rate of turnover of the respective compound but its level will not necessarily deviate from the initial value.

The glycogen content of the epithelium decreases at a rate of about 25 micrograms per hour. In 20 hours about 50% of the initial content has disappeared. Approximately the same rate of disappearance has been found for the liver of rat and dog, whereas in the liver of the pigeon as much as 75% can be recovered after incubation for 72 hours (18).

In tissues of animals, glycogen is supposed to be converted by phosphorolysis (19) into hexose-1-phosphate, which in turn is transformed into hexose-6-phosphate and then to hexosediphosphate. The phosphorolysis is not inhibited by iodoacetate (20), and in the presence of this substance the glycogen of the corneal epithelium disappears at the same rate as in controls. However, if iodoacetate is added, an increase of hexosediphosphate (DP) fraction is observed and the increase is greater when inorganic phosphate is added. This effect could be explained by the well known inhibition of triosephosphate dehydrogenase by iodoacetate and the consequent displacement to the left of the enzymatic equilibrium:



The undiminished rate of disappearance of glycogen and the simultaneous increase of slowly hydrolizable phosphate esters after addition of iodoacetate seemed to corroborate the assumption of a phosphorolysis. We failed, however, to demonstrate an inhibition of the glycogen disappearance by phloridzin, which is supposed to inhibit the phosphorolysis (21). This result cannot be regarded as a serious objection against phosphorolysis, since it has not been demonstrated that the corneal epithelium is sufficiently permeable for this inhibitor.

Glucose does not disappear in the presence of iodoacetate which would indicate that under these circumstances phosphorylations which require energy do not occur to any large extent. It is therefore, possible that the entire excess of phosphate in the DP fraction is hexosemonophosphate and derived from the phosphorolysis of glycogen and not diphosphate, the esterification of which would require, at least indirectly, oxidative energy. With this assumption, the appearance of an excess of 20 micrograms phosphorus in the DP fraction should

correspond to about 120 micrograms of hexose. We found, however, a disappearance of 200–250 micrograms of glycogen during the same time interval. This difference could possibly be explained by the assumption that only a part of the hexosemonophosphate is split up under our conditions of hydrolysis, which is possible according to Lohman's data. Alternative possibilities for the removal of glycogen are its enzymatic hydrolysis or a direct oxidation of hexosemonophosphate. Neither one of these hypothetical pathways is sufficiently well known to make a discussion here profitable. The investigations on oxidation of hexosemonophosphate have recently been reviewed by Krebs (22).

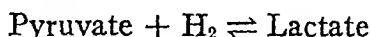
Glucose is rapidly metabolized in the supravital cornea. About 1000 micrograms disappear in the course of 12 hours. This disappearance is practically completely inhibited after addition of iodoacetate. By the inhibition of triosephosphate dehydrogenase and by the consequent suppression of the oxidation of triosephosphate and its breakdown products, iodoacetate prevents phosphorylations which require the supply of oxidative energy. The phosphorylation of glucose is one of such reactions and it is, therefore, understandable that in the presence of iodoacetate no hexosephosphate accumulates in excess of the amount which is found in the absence of glucose and that the disappearance of glucose is inhibited. Since no oxidative energy is required for the phosphorylation of glycogen, the formation of hexosephosphate from this precursor remains undisturbed by the addition of iodoacetate. The coupling of oxido-reductions with phosphorylation has been discussed in detail in two extensive reviews (16, 23).

Fluoride inhibits the disappearance of glucose to a lesser degree than does iodoacetate. In agreement with the results of other authors, it seems likely that enolase in our tissue preparations is only partially inhibited by fluoride (24).

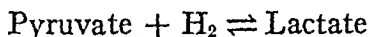
The influence of glucose on the lactic acid level is demonstrated by the high lactate content of the fresh cornea immediately after interruption of the natural glucose supply; by the maintenance of this initial value by irrigation or injection of glucose; and by the restoration of the considerable rate of disappearance of lactate if iodoacetate is present in addition to glucose.

In the conventional scheme for the carbohydrate breakdown, lactate is formed by the reduction of pyruvate. According to the scheme proposed by Ball (25), pyruvate can compete for the hydrogen of reduced diphosphopyridine-nucleotide (DPN) with those "hydrogen carrying" systems which eventually reduce molecular oxygen. If the rate of reduction of DPN exceeds the capacity of the intermediate systems which react with oxygen, pyruvate substitutes as oxidant and is reduced, with formation of lactate. Lactate, on the other hand, is oxidized to pyruvate if sufficient oxygen is available or if the rate of reduction of DPN by other competing systems is diminished.

In vivo, glucose is constantly supplied to the epithelium, the diphosphopyridine-nucleotide (DPN) is presumably reduced at a high rate, and the reaction:



moves to the right. The same will be the case in vitro, if glucose is supplied by injection or by irrigation. If iodoacetate is added, the oxidation of triosephosphate and the simultaneous reduction of DPN is inhibited, the consumption of glucose is prevented, no pyruvate is formed from glucose, and the oxygen supply will be ample to oxidize small amounts of DPN which are being reduced by other sources. The reaction:



will then be displaced to the left.

In this discussion, three oxidative pathways are discernible which can participate in the regulation of the lactic acid level. The first is the oxidation of DPN, which is reduced by triosephosphate; the second is the system which oxidizes pyruvate; the third is the oxidative mechanism for the oxidation of DPN, which is reduced in the transformation of lactate to pyruvate. Within the structural setup of the intact cell, the first and last pathway might or might not be identical.

The total reserves of carbohydrates and carbohydrate derivatives which can be oxidized by the excised cornea consist of glycogen, glucose, and lactate. In one hour one cornea has an oxygen uptake of about 70 cu. mm. (32°C.) or about 3.1×10^{-3} mM O_2 . In the same time interval the cornea consumes about 100 micrograms of glucose. The figures match exactly since for the complete oxidation of 100 micrograms of carbohydrate 73 cu. mm. O_2 are required. How-

ever, the small amount of endogenous glucose in the cornea is exhausted in 1-2 hours, and thereafter the carbohydrate consumption amounts to 25 micrograms glycogen plus 25 micrograms lactate. The remaining lactate disappears in about 12-20 hours. A deficit of oxidizable carbohydrates should ensue as soon as glucose has disappeared, this deficit becoming more marked after exhaustion of the lactate storage, since the rate of oxygen uptake is maintained at the initial level for at least 24 hours. This would indicate that as long as glucose is supplied to the cornea carbohydrate breakdown suffices for the maintenance of the oxidations, and in vivo the supply of glucose from the tears and through the limbal capillaries should prevent a deficit of oxidizable carbohydrate derivatives. Although the disappearance of lactate and glycogen is suppressed in the presence of an excess of glucose, the rate of glucose utilization is sufficiently fast to provide the required amount of oxidizable substrate. Once the glucose is exhausted, as is the case in the excised cornea after one or two hours, one half of the oxidizable substrate and, after disappearance of lactate, three fourths of the oxidizable substrate have to be derived from other sources than carbohydrates, presumably from fats or proteins.

The fact that the rate of oxygen uptake is maintained in vitro, possibly at the expense of material other than carbohydrate, could explain the fact that inhibitors of glycolysis, like iodoacetate, do not completely suppress the rate of respiration in this tissue (26).

The above considerations possibly provide a clue for the explanation of the effect of iodoacetate on the lactate disappearance. We observed in our experiments that the normal rate of lactate utilization in the excised cornea is retarded to a very slight extent by iodoacetate. In the presence of glucose or fluoride, or on incubation at a lower temperature, the lactate disappearance is practically completely inhibited, and is restored under these conditions by iodoacetate to almost the normal rate.

From the calculations in the previous paragraphs we have arrived at the conclusion that in the excised cornea glycogen and lactate together do not provide sufficient substrate for the saturation of the oxidative mechanisms. Therefore, the lactate oxidation can proceed at a maximum rate. However, when glucose is present and the reduction of oxidizable coenzyme by triosephosphate dehydrogenase

is accelerated, or when the oxidations are inhibited to a greater extent than the glycolytic mechanism, the triosephosphate dehydrogenase will compete for oxidized coenzyme with the lactic acid dehydrogenase and the lactate disappearance will be inhibited. In both instances iodoacetate should restore lactate consumption to about the normal rate since the competitive reduction of oxidized coenzyme by triosephosphate dehydrogenase is eliminated.⁵

Another aspect of the oxidation of lactate in the excised cornea should be discussed here. Our experiments show that lactate production from carbohydrate precursors takes place at a comparable rate in both the epithelium and in the stroma. The systems for enzymatic oxidation, however, are restricted to the epithelium, while the metabolism of the separated stroma is essentially anaerobic. In the fresh cornea three fourths of the lactate depot in the cornea are found in the stroma and the lactate content in the denuded stroma remains practically unchanged for as long as 20 hours. In the whole cornea, with the epithelium covering the stroma surface, the lactate depot in the stroma is depleted at a rate of 25 micrograms per hour, as mentioned above. Lactate from the stroma is, therefore, utilized by the epithelium and oxidized there through the cytochrome oxidase system, the presence of which in this tissue component has been demonstrated in a previous paper (1). The dependence of the lactate level in tissues upon oxygen supply is known as the "Pasteur effect". In our case the lactate level is regulated by the metabolic interdependence of the two histological components of a tissue and one could speak, therefore, of a "histological Pasteur effect". Such metabolic interaction might be of significance in many cases where stroma and epithelium form the main histological elements in an organic unit. The unequal distribution of oxidative enzymes in some tissues supports this suggestion. Lactate transfer and oxidation need not be the only metabolic interaction between an oxidatively very active tissue and an adjacent tissue with anaerobic metabolism. Studies previously published from this

⁵ We believe that it is permissible to omit in this discussion the role of the oxidation of pyruvate itself because this substance never accumulates in the cornea. When injected in large excess it is removed at an extremely fast rate of about 400 micrograms per hour and per cornea, by a non-oxidative mechanism which will be described in greater detail in one of the following papers (27).

laboratory indicate that in the ciliary body (28) and in the choroid plexus (29) an actual oxidative interchange takes place between epithelium and stroma through the mediation of a system of oxidation-reduction carriers. The more highly integrated economy resulting from such specific and extensive interactions may be imagined as one of the characteristics of tissue organization.

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XI. THE EFFECT OF MUSTARD ON SOME METABOLIC PROCESSES IN THE CORNEA*

HEINZ HERRMANN AND FAY H. HICKMAN

In the preceding paper we have reported our preliminary studies on the metabolism of the cornea, studies which enabled us to establish a crude balance between the rate of reaction in the glycolytic pathway and the rate of oxidation of the various oxidizable carbohydrate breakdown products. We were, consequently, in a position to test whether any of the metabolic pathways so far disclosed were affected by mustard. Previous experiments (1) on the loosening of the corneal epithelium by mustard had indicated that there might be a connection between metabolic oxidations and the development of the lesion. We were particularly interested, therefore, in finding out whether doses of mustard which produce this lesion also produce a recognizable disturbance in the metabolism, and whether or not such disturbances preceded the development of the lesion.

TECHNIQUE AND RESULTS

The exposure of the tissue to mustard and the supravital maintenance of the cornea have been described before (1). The biochemical techniques are given in the preceding paper (2). The experiments were performed on beef corneas.

(1) *Oxygen Uptake*. Our results clearly indicate an inhibition by mustard of the oxygen uptake of the cornea (Table I). The inhibition first appears after 3-4 hours of incubation. Exposure to mustard vapor¹ for 5 to 20 minutes and subsequent incubation at 32°C for 5 to 20 hours prior to measurement in the Warburg apparatus revealed a decrease in the oxygen uptake of about 25%, and this decrease remained fairly constant over a relatively wide range of experimental conditions. A more complete inhibition of oxygen uptake (50% or

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

¹ Under the conditions of our experiments the mustard uptake was estimated as 0.6 micrograms per cm² per minute.

more) was found after exposure to mustard for 60 minutes. It should be noted that control corneas incubated for as long as 19 hours showed some increase of O_2 uptake when compared with fresh tissue or with samples incubated for shorter periods. The onset of this enhanced O_2 uptake appears to coincide with the depletion of the lactate reserves noted in the previous paper and is, no doubt, associated with the oxidation of non-carbohydrate components of the tissue.

(2) *Glycogen*. There is a slight transitory increase in the rate of glycogen consumption during the first few hours after exposure to mustard. Thereafter no significant difference in the rate of glycogen

TABLE I

Effect of Mustard on the Oxygen Uptake, Anaerobic Acid Production and Lactic Acid Content of Beef Corneas

Incubation: 19-22 hours at 32-33°C.

The figures given each represent the average of 3-15 samples.

EXPOSURE TO MUSTARD, MINUTES	O_2 UPTAKE PER CORNEA CU.MM. PER HOUR	% INHIBITION O_2 UPTAKE	ANAEROBIC CO_2 OUTPUT CU.MM. PER CORNEA PER HOUR	LACTIC ACID CONTENT MICROGMS. PER CORNEA
0	84		67	30
5	54	27	77	137
0	73		86	69
20	45	25	87	445
0	83		76	85
120	40	52	52	455

consumption was noted between controls and mustard treated corneas. In the presence of excess glucose, neither controls nor mustard treated tissues showed a reduction in their glycogen content (Table II).

(3) *Anaerobic Glycolysis*. Anaerobic acid production was tested on tissues exposed to mustard, incubated in the presence of oxygen for various periods, and then suspended in the Warburg apparatus in Ringer's bicarbonate solution in an atmosphere of 95% N_2 and 5% CO_2 . The preliminary aerobic incubation was used in order to avoid the possibility that the development of the mustard injury might be inhibited by anaerobiosis, as we had found to be the case in respect to the loosening of the corneal epithelium. No difference was found

between tissues exposed to moderate doses of mustard and controls (Table I). Confirmatory evidence that the earlier steps in the glycolytic mechanism are not abnormal after exposure to mustard is found in the fact that iodoacetate, used in addition to mustard, gives the same inhibition of glucose consumption and the same increase in the hexose phosphate fraction (Table VI) as it does in the absence of mustard.

(4) *Lactate Utilization.* By far the most pronounced metabolic effect which we observed in the cornea on exposure to mustard is an inhibition in the lactate disappearance (Table III). Since there was no parallel increase in the rate of consumption of lactate precursors

TABLE II

Glycogen Content of Corneal Epithelium after Exposure to Mustard

The figures given each represent the average of 3-15 samples.

Incubation at 32°C.

	CONTROL	EXPOSED
Exposure 10 minutes		
Incubation time		
10 hours	926	799
20 hours	589	519
Exposure 15 minutes		
Incubation 20 hours	783	722
+ 2 mg. glucose	1052	1106

(glucose and glycogen), we concluded that the failure of the lactate reservoir to become depleted on incubation was due to an inhibition in the utilization of lactate, not to an enhanced rate of production. If iodoacetate is applied in addition to mustard, the lactate utilization is reestablished, though at somewhat slower rate than in the absence of mustard. The interpretation of this effect will be discussed in a subsequent paper (3).

Exposure of the cornea to mustard vapor for 3 minutes results in a slight inhibition of lactate disappearance (Table IV). After exposure of 5 minutes the rate of lactate disappearance was reduced by somewhat more than 50 %. Exposures of 15-20 minutes brought the disappearance of lactate to a complete standstill. Since in normal

controls there is a measurable loss of lactate on incubation for 2 hours, our failure to detect any loss of lactate following exposures of these

TABLE III

Lactic Acid Content of Beef Corneas. Effect of Mustard, Iodoacetate, and Mustard + Iodoacetate

Exposure to mustard vapor: 5-10 minutes.

Incubation: 12 hours at 32°C.

MICROGRAMS LACTIC ACID PER CORNEA			
Control	Iodoacetate .5 mg. per cornea	Mustard	Mustard plus Iodoacetate
80	150	360	
90		360	
60		240	
70		330	
160		250	
80	130	300	210
100	90	360	180
60	80	310	220
60	110	270	170
110	160	410	280
170	150	440	150
	250	300	220
		330	260
Av. 93	140	328	220

TABLE IV

*Effect of Mustard on Lactic Acid Content of Beef Corneas.
Variation in Exposure Time*

Incubation: 10 hours at 32°C.

MICROGRAMS LACTIC ACID PER CORNEA			
EXPOSURE IN MINUTES	WHOLE CORNEA	EPITHELIUM	STROMA
0	170	22	36
3	185	15	85
5	240	45	260
10	315		

magnitudes indicates that the lag period in the onset of inhibition of lactate utilization following exposure to mustard is less than two hours.

TABLE V

Effect of Mustard on Extractable Phosphate Fractions

Incubation time: 10-12 hours at 32°C.

MICROGRAMS PHOSPHORUS PER CORNEA

IP			ATP			DP			PG		
Control	5' Treatment	10-20' Treatment	Control	5' Treatment	10-20' Treatment	Control	5' Treatment	10-20' Treatment	Control	5' Treatment	10-20' Treatment
22		23	24		27	25		14	34		38
20		25	14		23	22		13	33		43
26		27	13		17	24		35	31		41
30		28	15		23	34		31	32		37
21		33	17		26	37		22	38		44
31		32	18		27	25		18			42
19		25	15		27	22		14			
19		24	19		28	20		13	32		47
24		33	28		23	14		11	38		40
25		31	15		32	25		4	42		39
25		28	21		28	17		11	34		34
26		30	14			17	15		19		25
22	22	28	12	29	22	23	15	26	36		33
17	25	27	15	23	27	25	24	6	29		40
19	25	25	15	17	28	22	21	7	28		32
19	26	25	13	20	31	23	17	7	28		25
23	28		14	28		28	24		22	38	35
25	28		23	18		20	23		30	35	37
24	23	24	26	19	23	20	20	23	34	33	42
	23			24			18			36	
23	22	21	19	22	25	23	20	22		34	40
	23			21			22			36	
25		27	17		13	24		34			
23		36	18		18	24		18	27		45
24		30	12		28	21		22	35		41
28		31	16		17	21		34			
22		29	18		22	23		26			
25		28	20		30	18		17			
22		22	25		30	22		16			
27		31	26		32	17		22			
21		24	22		26	20		25			
21		25	18		24	23		22			
22		26	19		28	20		22			
25		30	17		25	27		21			
Av. 23	24	28	18	22	25	23	19	19	32	35	40

Total Extractable Phosphate..... Control 5' Treatment 10-20' Treatment

Micrograms Phosphorus per Cornea..... 96 100 112

IP = Phosphate determined directly i.e. inorganic phosphate + creatine phosphate + acetyl phosphate.

ATP = Phosphate determined after 7 minutes hydrolysis with N/1 HCl minus IP, i.e. chiefly adenosine triphosphate.

DP = Phosphate determined after 180 minutes hydrolysis with N/1 HCl minus (IP + ATP), i.e. chiefly hexose diphosphate.

PG = Phosphate determined after digestion with perchloric acid minus (IP + ATP + DP), i.e. chiefly glycerophosphate.

This effect is, therefore, among the earliest of the reactions to mustard so far recognized.

These results are to be compared with those of our experiments previously reported on the loosening of the corneal epithelium following

TABLE VI

Extractable Phosphate Fractions. Effect of Mustard, Iodoacetate, and Inorganic Phosphate

Exposure to mustard vapor: 10 minutes.

Incubation: 10-12 hours at 32°C.

Iodoacetate injected 0.5 mg. per cornea.

Sodium phosphate injected 0.1 mg. per cornea.

MICROGRAMS PHOSPHORUS PER CORNEA						
Phosphate Fractions	Control	Iodoacetate	Phosphate	Iodoacetate + Phosphate	Iodoacetate + Mustard	Iodoacetate + Mustard + Phosphate
IP	22	13			17	
	25	15			11	
	20	12				
	26	13				
ATP	20	7	22	10, 0	2	10
	21	9	25	15, 5	9	0
	15	15	26	11		5
	19	16	23	15		8
DP	25	40	16	85, 98	53	82
	25	54	21	59, 63	45	82
	22	42	28	51, 63		76
	20	42	35	85		78

IP = Phosphate determined directly i.e. inorganic phosphate + creatine phosphate + acetyl phosphate.

ATP = Phosphate determined after 7 minutes hydrolysis with N/1 HCl minus IP, i.e. chiefly adenosine triphosphate.

DP = Phosphate determined after 180 minutes hydrolysis with N/1 HCl minus (IP + ATP), i.e. chiefly hexose diphosphate.

exposure to mustard. Under the conditions of our experiments this symptom was just detectable on incubation following exposure of 5 minutes, and was maximal following exposures of 15-20 minutes. The first appearance of the symptom occurs after 5-6 hours of incubation; that is, after the onset of the inhibition of lactate utilization.

If there is a causal connection between these two phenomena, the inhibition of lactate utilization is the cause of the loosening, not the reverse.

(5) *Phosphate Fractions.* A study of the phosphate fractions showed a slight but definite increase in the total extractable phosphate (Tables V, VI). In a previous paper (1) it was noted that mechanical separation of the epithelium from the stroma also results in an increase in the extractable phosphate. One may raise the question whether the loosening of the corneal epithelium by mustard similarly activates a system for the mobilization of soluble phosphate.

DISCUSSION

The major effects of mustard injury disclosed in these experiments are an inhibition of the utilization of lactate and a decrease in the oxygen uptake, the decreased oxygen uptake being approximately equivalent to that required for the complete combustion of the unutilized lactate. These metabolic changes are found within the same dosage range as that required for the loosening of the corneal epithelium and occur in advance of the first noticeable manifestation of loosening. There is no proof that these two phenomena are causally connected. In the present discussion, we wish merely to analyze what interpretations would be imposed on our various findings by the acceptance of the notion that the metabolic changes which we have found, and the subsequent loosening of the corneal epithelium, are causally connected.

In the study of the loosening of the corneal epithelium it was shown that under anaerobiosis the loosening does not occur. Our present findings, that the oxygen uptake of mustard treated tissue is inhibited in proportion to the reduced consumption of lactate, present an apparent paradox for there appears, at first sight, to be no oxidation left over to account for the loosening of the epithelium. The onset of the decline in O_2 uptake, however, apparently lags behind the decline in lactate consumption by an interval of one or more hours. Since the time of onset of the inhibition of lactate utilization and of decreased O_2 uptake cannot be specified exactly, the magnitude of the lag between them cannot be estimated. The difference may, however,

be sufficient to account for the oxidation of appreciable cellular material. The fact that this excess of oxygen uptake over the apparently available substrate eventually stops may be taken to indicate that some oxidizable component finally gets used up in the process.

In the normal cornea after incubation for 15–20 hours the lactate reservoir is depleted, but the oxygen uptake in the normal tissue does not decline as it does after the utilization of lactate is inhibited by mustard. On the contrary, there is frequently a rise in O_2 uptake, and we have attributed this rise, and the absence of a fall, to the oxidation of non-carbohydrate metabolites. It may be noted that some partial loosening of the epithelium occurs under these conditions. Nevertheless one may well ask—how is the failure of utilization of such alternative substrates to be explained in the case of mustard injury? It may be supposed that the hydrogen transport system which links the oxidation of lactate to the reduction of molecular oxygen contains many steps. The failure of the system to oxidize alternative substrates when lactate cannot be utilized indicates that the inhibition produced by mustard may not affect specifically the lactic acid dehydrogenase, but rather some enzyme system higher up in the chain. On the other hand the fact that some oxidation (50–70% of normal) goes on even when the utilization of lactate is completely inhibited indicates that the hydrogen transport system for lactate and its alternates is a special one, distinct from that involved in the rest of the respiratory process. Glucose and glycogen are, for instance, utilized at a normal rate in mustard treated corneas, and their utilization is not associated with an extra accumulation of lactate except under anaerobiosis.

Our experiments, therefore, suggest that in the cornea there is a special hydrogen transport system for at least a major part of the lactate oxidation, that this transport system is damaged by mustard, and that, as a consequence, some component of this system, which normally is kept chiefly in its reduced state by the oxidation of lactate or its alternates, becomes oxidized. The hypothesis that these metabolic events are connected with a loosening of the epithelium requires the more specific assumption that the oxidation of this intermediate is the cause of the loosening, because on incubation under nitrogen the loosening does not occur.

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XII. FURTHER EXPERIMENTS ON CORNEAL METABOLISM IN RESPECT TO GLUCOSE AND LACTIC ACID*

HEINZ HERRMANN AND FAY H. HICKMAN

I. GLUCOSE CONSUMPTION AND LACTATE PRODUCTION

In preceding papers we have been dealing with some reactions of the endogenous carbohydrate metabolism in the excised surviving cornea. In particular, we followed the depletion of the stores of glycogen and of lactate, and observed that the lactate utilization is almost completely blocked by mustard within a short time after exposure.

There is only a small quantity of glucose in the fresh cornea and this amount disappears within the first two hours of incubation, while the stores of glycogen and of lactate are not exhausted for 12 and 20 hours respectively. It follows that in the case of the excised cornea the maintenance in the normal state for as long as one day, or the development of the pathological lesion (loosening of the epithelium) which does not appear before 5 hours after exposure to mustard, cannot be connected with the normal or disturbed metabolism of glucose. Although glucose metabolism could not, therefore, be of direct relevance for the maintenance and pathology of the excised cornea during this time interval, an examination of glucose utilization in the cornea was nevertheless desirable. In vivo, glucose is constantly supplied to the cornea by the tears and through the limbal capillaries. Moreover, our previous experiments have shown that externally supplied glucose is utilized, and that in the presence of glucose the lactate and glycogen reservoirs in the tissue are kept filled. Therefore, experiments were carried out in which the utilization of glucose was tested more directly both in the whole cornea and in the separated stroma, in normal samples as well as after exposure to mustard. By separating these experiments on the utilization of externally supplied glucose from our previous data on utilization endogenous materials, we hoped to gain some clarity in the presentation of the problem.

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

RESULTS AND COMMENTS

The experiments were carried out on beef corneas as described in the preceding papers. The anaerobic acid production of normal and mustard treated corneas was tested in the Warburg apparatus with and without added glucose after 20 hours of incubation of the tissue in a moist chamber at 29°C. At this time all endogenous stores of glucose have been completely exhausted and any effect of mustard on the metabolism should be fully developed. It was found (Table I) that in the absence of added glucose the anaerobic acid production of the controls and mustard treated tissues was the same, thus confirming

TABLE I
*Manometric Determination of Anaerobic Acid Production by Beef
Corneas after Preliminary Incubation
(20 hours at 29°C.)*

CO ₂ PRODUCTION IN CU.MM. PER CORNEA PER HOUR			
Controls	Controls + Glucose	Exposed	Exposed + Glucose
90	204	102	143
92	191	98	150
80	194	78	120
89	185	75	125
92	165	95	135
98	168	103	128
82	170	90	149
84	178	100	145
Av.....88	181	93	137

our previous conclusions that mustard does not affect the glycogenolytic processes in the tissue except for a slight acceleration of glycogen utilization during the first few hours after exposure. In the presence of added glucose there is a great increase in anaerobic acid production, but this increase is significantly less in the mustard treated tissue than in the controls. It is plain that a late effect of mustard is an injury to a part of the glycolytic mechanism. The experiments which follow show somewhat similar effect observable also under aerobic conditions. In these experiments glucose was injected into the stroma of control and mustard treated corneas. The glucose and lactic acid content

were estimated chemically on some samples before incubation, on others after incubation for 10 hours (Table II). The glucose consumption of the control and mustard treated corneas was essentially equal, but the lactate reservoir rose to slightly higher levels in the controls than in the treated corneas. Since in the mustard treated corneas the utilization of lactate is, as we already know, markedly reduced, the difference in the rise of the lactate level is probably significant.

Similar experiments performed on the denuded stroma (Table III) showed that glucose consumption and lactate production ceases in the

TABLE II
*Glucose Utilization and Lactate Production in Excised Corneas,
Normally and after Exposure to Mustard*

Incubation time: 10 hours at 32°C.

2 mgms. of Glucose per cornea. Two corneas per sample.

GLUCOSE CONTENT IN MICROGRAMS PER CORNEA			LACTATE CONTENT IN MICROGRAMS PER CORNEA		
Before incubation	After incubation		Before incubation	After incubation	
	Controls	Exposed		Controls	Exposed
1392	347	462	450	750	605
1419	351	523	420	620	530
1816	560	616	316	506	516
1780	490	530	328	483	466
Average.1602	437	533	379	590	529
Glucose consumed.	1165	1069	Increase in Lactate. . . .	211	150
Consumed per hour. . . .	117	107			

denuded tissue after 4 to 5 hours of incubation. During the first three hours the glucose consumption is about 100 micrograms per cornea per hour, practically equal to that of the whole cornea. Only about half of the glucose lost is recovered as lactate. No difference was found between controls and mustard treated tissues.

Since the stroma has no oxygen uptake, our failure to recover all the lost glucose as lactate required explanation. Studies were therefore made on the extractable phosphate of the tissue. Experiments previously reported have shown that, when whole corneas are incubated, no changes occur in the phosphate fractions determined on the epithe-

lium and stroma separately. It was found, however, that after incubation of the denuded stroma there is a very marked increase in the least easily hydrolyzable phosphate fraction (mainly glycerophosphate) without any change in the total extractable phosphate. The increase occurs chiefly at the expense of inorganic phosphate which is almost completely used up in the process. The increase in slowly hydrolyzable phosphate amounted to 20–30 micrograms phosphorus per

TABLE III

*Glucose Utilization and Lactate Production in the Isolated Stroma
with and without Exposure to Mustard*

Exposure time: 15 minutes. Preliminary maintenance at room temperature.
Incubation at 32°C.

PRELIMINARY MAINTENANCE	INCUBATION AFTER INJECTION	GLUCOSE UTILIZATION IN MICRO- GRAMS		LACTATE INCREASE IN MICRO- GRAMS	
		Control	Exposed	Control	Exposed
<i>hrs.</i>	<i>hrs.</i>				
3	3½	310	340	24	20
		380	280	124	119
4	4	410	400	80	80
		290	340	120	95
5	5	360	380	200	210
		550	580	205	220
2	8	430	415	210	230
		475	450	243	238
2	8	440	505	168	179
		450	390	205	212

cornea. If this is counted as glycerophosphate it accounts for the major part of the difference between glucose lost and lactate recovered. It appears, therefore, that the denuded stroma stops utilizing glucose because it is unable to transfer phosphate from glycerophosphate to glucose. This transfer requires the presence of carriers of energy-rich phosphate bonds. In the absence of the epithelium these carriers apparently are gradually lost. Presumably the epithelium normally assists the stroma in the production of such phosphate carriers.

In the intact mustard treated cornea no such marked change is found in the extractable phosphate fractions of the stroma. It is to be concluded, therefore, that the decrease in glucose consumption, which sometimes occurs after exposure to mustard, is probably not due to the same mechanism as that which occurs in the denuded stroma. It might be due to an inhibition of hexokinase, which Dixon (1) has shown to be specially susceptible to mustard injury. An examination of Table IV shows that the the mustard effect on glucose consumption does not occur regularly, but depends in large measure on the conditions of incubation following exposure to mustard. The fact that the loss of glucose consuming power in the denuded stroma is no more rapid

TABLE IV
*Effect of Mustard upon Glucose Utilization under
Varying Experimental Conditions*

EFFECT OF MUSTARD ON GLUCOSE UTILIZATION	TISSUE	GAS PHASE	SUBSTRATE SOLUTION SUPPLIED
No inhibition	Whole Cornea	O ₂	Injection of glucose in Ringer's
No inhibition	Whole Cornea	N ₂ + CO ₂	No external substrate suspended in Ringer + bicarbonate
No inhibition	Stroma	O ₂ (no uptake)	Injection of glucose in Ringer's
Inhibition (30%)	Whole Cornea	N ₂ + CO ₂	Suspended in glucose solution in Ringer + bicarbonate

after mustard treatment than in the controls suggests that this mustard effect on the glucose metabolism may be located in the epithelium rather than in the stroma.

II. LACTIC ACID CONSUMPTION

In the experiments reported above it was found that the freshly denuded stroma can consume glucose at a rate of 100 micrograms per hour equal to that of the whole intact tissue. One might conclude that in the presence of a constant supply of glucose practically the whole carbohydrate requirement of the epithelium might be met by lactate supplied from the stroma, and that the epithelium consumes little or no glucose directly. This, however, seems not to be true, for if lactate

is injected into the stroma and the whole cornea incubated without added glucose, the lactate is consumed at a rate of 25 micrograms per hour; that is, at precisely the same rate at which the normal endogenous reservoir of lactate is depleted in the absence of added substrate. It is to be concluded that this is the maximum rate at which lactate can be transferred to or consumed by the epithelium. Since in the presence of abundant glucose the lactate reservoir rises only slightly, the stroma in the intact cornea cannot be utilizing glucose at a rate

TABLE V

Comparison of Disappearance of Endogenous and Injected Lactate in Control Corneas and after Exposure to Mustard

Exposure to mustard: 10 minutes.

Incubation: 10 hours at 32°C.

Three corneas per sample.

	MICROGRAMS OF LACTATE PER CORNEA		
		Average	Estimated Lactate Consumed
Before Incubation:			
Uninjected	373, 380	376	
Injected	1330, 1290	1310	
After Incubation:			
Controls:			
Uninjected	66, 105	86	290
Injected	1050, 1040	1045	265
Exposed to Mustard:			
Uninjected	386, 253	319	57
Injected	1253, 1200	1226	84

much greater than 25 micrograms per hour. When the epithelium is removed the rate of glucose utilization in the stroma increases three-fold. Returning to the mustard problem, we found that exposure to mustard inhibits the utilization of injected lactate in the same degree as it inhibits the utilization of endogenous lactate (Table V).

Our previous analysis has shown that approximately three fourths of the lactate reservoir in the fresh cornea is to be found in the stroma. Though the amount in the epithelium represents a small fraction of the total, the concentration of lactate in the epithelium is about twice

that in the stroma. It is not clear whether the epithelial lactate is derived in whole or in part from the stroma supply, and whether part of the epithelial lactate reserve, which accumulates in the presence of an abundant supply of glucose, may be due to a bottle neck in the hydrogen transport system of the epithelium, which permits some lactate to be formed by glucolysis in excess of the rate of oxidation. The possible competition of various oxidizable substrates for a limited supply of oxidized coenzymes has already been discussed in a previous paper.

Distinct from the question of the origin of the epithelial lactate is the further question of the utilization of lactate by the epithelium. In order that the epithelium may utilize the stroma lactate, the transport of stroma lactate to epithelium, either by diffusion or by active transfer is required. Furthermore, the utilization of the stroma lactate by the epithelium may involve mechanisms distinct from those available for use of endogenous lactate within the epithelium. Since the utilization of lactate is inhibited by mustard it was possible to attack this question experimentally, and to test whether the utilization of lactate from the two sources is equally affected by mustard injury.

In a preliminary series of experiments the corneas, after exposure to mustard, were incubated for varying periods. The epithelium was then scraped off and the lactate content of the separated tissues estimated separately. The results were compared with fresh uninjured tissues and with control samples not exposed to mustard but incubated for the same period.

In control samples the epithelial lactate reservoir drops sharply during the first hour (Table VI). The stroma lactate remains unchanged during this period. Whether this is because no stroma lactate is transferred to the epithelium during this time, or because the rate of production of lactate from the rapidly exhausted endogenous glucose reserve in this tissue just equals the loss, is not clear. Since the fresh stroma contains glucose and under special experimental conditions has been shown capable of utilizing it, one would be inclined to assume that the latter is the case. About two hours after incubation a beginning depletion of the stroma lactate reserve becomes measurable. This coincides with the time of depletion of endogenous glucose. Also, it coincides with the time when the lactate concentration in the epithelium has fallen approximately to that in the stroma.

In mustard treated tissues the consumption of the epithelial lactate proceeds at an essentially normal rate during the first two hours, but with adequate dosage the utilization of stroma lactate is completely inhibited. It is not immediately clear whether the preliminary consumption of part of the epithelial lactate occurs because the mustard effect has not had time to develop, or because the epithelial mechanism for consumption of endogenous lactate is less sensitive to mustard injury. With more prolonged incubation the lactate content of the epithelium in mustard treated tissues declined no more rapidly than in controls, in spite of the fact that in the mustard treated samples no further supply of stroma lactate was available. It follows that the

TABLE VI
Disappearance of Lactate from the Epithelium of the Cornea
Incubation of whole corneas. Removal of Epithelium immediately before Lactate determination. Four corneas per sample.

LENGTH OF INCUBATION hours	LACTATE CONTENT OF THE EPITHELIUM IN MICROGRAMS PER CORNEA				AVERAGE
0	107	80	95		94
1½	63	54			59
2	60	41			51
3	36	35			36
4	40	32	12		36
5	31	26			29
10	7	17		2	10

mechanism for consumption of endogenous lactate in the epithelium is damaged by mustard, but, since some lactate consumption continues in the epithelium even when the utilization of stroma lactate is completely inhibited, it is apparent that the two systems are not equally sensitive to mustard injury.

In order to examine these matters in greater detail we have sought experimental conditions under which an appreciable time interval could be interposed between the exposure to mustard and the onset of utilization of the lactate stores. It is possible to achieve this by keeping the cornea at a lower temperature or under anaerobiosis for a preliminary period following exposure to mustard. At the end of this preliminary period samples are assayed for their content of lactate

in epithelium and stroma while other samples are incubated aerobically to test the loss of lactate in the two tissues. Neither of these procedures can be regarded as leaving the tissue unaffected except in preventing the consumption of lactate. As a matter of fact, both procedures had previously been shown to suppress the pathological process of loosening

TABLE VII
Lactate Disappearance in Corneal Epithelium after Preliminary Maintenance at 0°C.

INCUBATION AT 0°C.	INCUBATION AT 29°C.	LACTATE CONTENT OF EPITHELIUM IN MICROGRAMS PER CORNEA			
		Before Incubation at 29°C.		After Incubation at 29°C.	
		Control	Exposed to Mustard	Control	Exposed to Mustard
<i>hours</i>	<i>hours</i>				
7	3	115	105	55	51
				57	55
2½	2½	96	90	53	52
				53	51
7	3	72	67	42	42
				40	50
6	2	84	87	42	42
				45	52
6	2	84	93	47	54
				42	53
6	2	107	95	49	55
Average.....		93	89	48	51
				(inhibition absent)	
1	10	420	LACTATE CONTENT OF WHOLE CORNEAS IN MICROGRAMS		
			410	120	360
				190	330
				(inhibition present)	

of the epithelium and even to reduce slightly the loosening of the epithelium which occurs on subsequent aerobic incubation. This complication is, however, not without advantage, for if the loosening of the epithelium is, as we have suggested, connected with the inhibition of lactate consumption, then we should find that mustard treated tissues subjected to these preliminary treatments would show less

inhibition of lactate utilization on subsequent aerobic incubations than is the case in tissues incubated aerobically directly after exposure.

Preliminary incubation at zero degrees revealed no effect (Table VII). The decline of lactate concentration in the epithelium during the first 2 to 3 hours of subsequent incubation at 29°C was the same as in the controls, while estimates of the lactate content of the whole cornea following ten hours of incubation at 29°C. showed that the

TABLE VIII

Lactate Content in the Epithelium after Preliminary Anaerobic Maintenance

Incubation at 29°C.

Epithelium of 4 Corneas per sample.

INCUBATION ANAEROBICALLY	INCUBATION AEROBICALLY	LACTATE CONTENT OF EPITHELIUM IN MICROGRAMS PER CORNEA			
		Before Aerobic Incubation		After Aerobic Incubation	
		Control	Exposed to Mustard	Control	Exposed to Mustard
hours	hours				
2½	2½	74	88	32	40
				29	50
5	2½	101	122	69	86
				50	94
4	2½	92	125	59	62
				63	71
2½	2½	70	95	25	34
				34	45
4	2½	105	120	55	69
				63	77
4	2½	110	139	71	105
				67	103
Average.....		92	115	51	70
		Lactate Consumed		41	45

stroma stores were completely intact. This result might perhaps have been predicted, for at zero degrees both the biochemical consequences of the combination of mustard with the tissue and also any recovery from the injurious effects should be almost completely inhibited.

Experiments in which there was a preliminary period of anaerobic incubation, however, afforded quite clear cut results. In the first

place, there was a marked decrease in the inhibition of lactate consumption on subsequent aerobic incubation. In the second place no inhibition in the consumption of the epithelial lactate reservoir was found in those experiments (Table VIII), and all the remaining inhibition had to be attributed to an injury in the mechanisms for consumption of stroma lactate (Tables IX, X). These experiments considered by themselves leave it still unclear whether the mechanism for consumption of endogenous lactate in the epithelium is less susceptible to mustard injury than the mechanism for transfer and consumption of stroma lactate, or whether the former merely recovers more com-

TABLE IX

Disappearance of Lactate from Whole Corneas after Exposure to Mustard and after Preliminary Anaerobic Incubation at 15°C.

LACTATE CONTENT IN MICROGRAMS PER CORNEA			
After Anaerobic Incubation at 15°C. for 10 hours		Anaerobic Incubation at 15°C. for 10 hours followed by Aerobic Incubation at 28°C. for 10 hours	
Controls	Exposed to Mustard	Controls	Exposed to Mustard
300	396	8	160
316	342	25	174
271	270	26	166
266	415	25	150
Av....288	331	21	162
	Lactate Consumed	267	169

pletely under anaerobiosis. Taken in connection with the experiments without preliminary anaerobic incubation, the former of these two possibilities seems more likely, but in any case, it is clear from these experiments that the two mechanisms are sharply distinguished.

The consideration of these experiments reveals one further minor point. After anaerobic incubation, the lactate level in the epithelium rises somewhat higher in mustard treated corneas than in the controls. This may be correlated with the increased rate of glycogen consumption during the first few hours after exposure to mustard noted in a previous paper. It is also to be correlated with the diminished total

lactate production in the whole cornea after exposure to mustard in the presence of glucose. We have previously suggested that this deficit in lactate production is attributable to a partial inhibition of glucolysis in the epithelium. With a decrease in the ability of the epithelium to utilize its endogenous glucose, the mustard treated tissue would be expected to begin consuming its endogenous glycogen sooner than the controls, and this is what we have found. It is likely, however, that in the presence of extra glucose the utilization of glycogen in the epithelium is suppressed.

TABLE X

Lactate Disappearance from Whole Corneas after a Preliminary Anaerobic Maintenance Period

Anaerobic: 10 hours; Aerobic: 10-12 hours.

Incubation Temperature 29°C.

LACTATE CONTENT IN MICROGRAMS PER CORNEA			
After Anaerobic Maintenance		After Anaerobic and Subsequent Aerobic Maintenance	
Control	Exposed to Mustard	Control	Exposed to Mustard
1100	1180	690	690
950	920	690	810
1150	1250	590	760
1480	1150	720	810
740	1000	550	620
1000	1000	650	700
Av.....1070	1083	648	732
Lactate Consumed		422	351

SUMMARY

The experiments reported here have added somewhat to our knowledge of the metabolic interactions between corneal stroma and epithelium. It was found not merely that the epithelium consumes the lactate produced by the stroma, but indirect evidence suggests that in addition the rate of glucolysis in the stroma is markedly influenced by the presence of the epithelium. In other tissues, the intracellular control of the rate of glucolysis and glycolysis by the activity of the respiratory system is well recognized, and has been called the Pasteur

effect. The corneal epithelium exhibits a typical Pasteur effect in that glycogen is consumed more rapidly under anaerobic than under aerobic conditions. In addition, it appears that in the cornea the interaction of epithelium and stroma involves processes similar to those concerned in the Pasteur effect. In the intact tissue it is estimated that about one quarter of the carbohydrate requirement of the epithelium is furnished in the form of lactate by the stroma.

At least one of the metabolic interactions between stroma and epithelium is profoundly damaged by mustard injury in doses which likewise lead to a loss of cohesion between the two tissues. Moreover, anaerobic incubation which diminishes the effect of mustard injury in respect to loosening of the epithelium also diminishes the effect in respect to this metabolic interaction. It may be suggested, therefore, that the metabolic interaction between stroma and epithelium is connected with the mechanism for the maintenance of the tissue cohesion, and that injury to this mechanism by mustard results both in the loss of cohesion and in the loss of metabolic interactions.

The mechanism by which the epithelium consumes its endogenous stores of lactate is distinct from that by which it acquires and consumes the lactate of the stroma, the latter being more susceptible to mustard injury than the former. The question as to whether the transport of lactate from stroma to epithelium involves an active transfer or simple diffusion will be discussed in a subsequent paper.

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XIII. THE CONSUMPTION OF PYRUVATE, ACETOIN, ACETATE, AND BUTYRATE BY THE CORNEA*

HEINZ HERRMANN AND FAY H. HICKMAN

In the preceding papers we have been concerned with the utilization of glucose, glycogen, and lactate by the cornea. Presumably the major oxidative pathways for the consumption of all three of these substrates pass through pyruvate. Nevertheless no appreciable quantities of pyruvate (or other keto acids) are to be found in the cornea. One would expect, therefore, that the utilization of pyruvate must be very rapid. In the present paper we shall report the results of our studies on the utilization of pyruvate which, in certain respects confirm this expectation. The latter experiments were not pushed to a final conclusion since it was found that exposure to mustard yields no detectable change in pyruvate consumption. A partial picture of the route of pyruvate utilization in the cornea was nevertheless achieved and will be reported here.

TECHNIQUE

Beef corneas were used in these experiments. The manipulation of the corneas and the technique of application of inhibitors and metabolites has been described in previous papers. The method of assay of lactate has also been reported previously.

Pyruvate was prepared from a fresh sample of Eastman's pyruvic acid by fractional distillation, careful neutralization of the proper fraction with sodium hydroxide, and precipitation of the sodium salt with alcohol. Determinations of pyruvate in trichloroacetic acid extracts of corneas were carried out according to Lu's method (1). Acetaldehyde was determined by the method of Stotz (2). CO_2 was measured manometrically as described below.

Acetate determinations were carried out as follows: 3 corneas were pooled for each sample and 8 ml. of $\text{N}/12 \text{ H}_2\text{SO}_4$ and 1 ml. of a 10%

* The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

solution of sodium tungstate were added. The samples were allowed to stand for about one half an hour and then ground in a mortar for 15 minutes. The extracts were separated from the insoluble residue, and to each sample 10 ml. of a saturated solution of NaH_2PO_4 , containing 1 ml. of concentrated phosphoric acid and 100 mg. of phenylhydrazine, were added. The mixture was placed in a vacuum still and the pressure reduced to 10–12 mm. Hg and steam slowly admitted. The connection of the pump was closed and the steam distillation continued for 20–30 minutes, the vacuum decreasing in this time to 50–60 mm. Hg. The distillate was titrated with 0.01 N NaOH using phenolphthalein as indicator.

Acetoin was determined by the method of Stotz and Rahberg (3). We used two corneas per sample. To these we added a sufficient amount of the tungstate sulfuric acid mixture to bring the volume to 10 ml. After standing at room temperature for at least 15 minutes the corneas were ground in a mortar and the solid particles removed by centrifugation. 2 ml. of the extract were used for conversion to diacetyl and half of this for distillation. 2 ml. of 2.5% bromine water were used for bromination. Octylalcohol, which is prescribed as a detergent to be used in the wash fluid, was not immediately available. We found that 0.02% zephiran¹ had the desired effect and did not interfere with the determination.

Butyric acid was determined by a method which followed in principle that of Bobbit and Deuel (4). Ten corneas were placed in 100 ml. of 0.1 N H_2SO_4 containing 1% sodium tungstate. After standing at room temperature for 15–20 minutes they were ground in a porcelain mortar without sand for at least 10 minutes. The fluid was poured into centrifuge tubes and 80 ml. of clear supernatant fluid was collected after centrifugation. This was transferred to an Erlenmeyer flask and 20 ml. of 10% CuSO_4 were added. Ten mg. of powdered $\text{Ca}(\text{OH})_2$ were added and the suspension was shaken vigorously every 10 minutes for half an hour. After this the sample was filtered and 50 ml. of the filtrate used for distillation. 100 ml. of N/1 H_2SO_4 were added and distillation performed until only about 10 ml. remained in the distilling flask. The distillate was transferred to a flask connected with a

¹ Zephiran is alkyl (C_8 — C_{18})-dimethyl-benzyl ammonium chlorid, Alba Pharm. Co.

reflux condensor. 100 ml. of van Slyke's mercury reagent were added to the distillate and the mixture was refluxed. When boiling commenced 10 ml. of a saturated potassium bichromate solution were added through the reflux condensor and the boiling was continued for 15 minutes. The fluid was then distilled off until 25-50 ml. remained

TABLE I
Utilization of Pyruvate by Beef Cornea

	AMOUNT OF PYRUVATE INJECTED	MICROGRAMS OF PYRUVATE RECOVERED PER CORNEA					
		Whole cornea			Stroma		
Immediately after injection	1 mg.	410 470	450 510	480 430	410 560	510	470
After incubation							
Time Temp.							
2 hr. 32°C.	1 mg.	210	210		490	480	
4 hr. 32°C.	1 mg.	125	115		370	390	
5 hr. 32°C.	1 mg.	100 120	105 110		390	480	
Immediately after injection	2 mg.	1535	1350				
After incubation							
Time Temp.							
4 hr. 29°C.	2 mg.	590	550				
Immediately after injection	4 mg.	3000					
After incubation							
Time Temp.							
2½ hr. 29°C.	4 mg.	1935	1930				

in the distilling flask. The distillate was aerated with CO₂ free air and titrated with 0.01 N NaOH using bromthymol blue as indicator.

RESULTS

1) *Pyruvate Consumption.* When sodium pyruvate is injected into the corneal stroma denuded of epithelium and tissue incubated practically all of the injected pyruvate can be recovered on subsequent extraction (Table I). It is not surprising that this tissue, which

has no measurable oxygen uptake, should fail to oxidize pyruvate, but it is rather remarkable that only small losses of pyruvate occur by dismutation or reduction.

When pyruvate is injected into the corneal stroma with the epithelium intact very rapid disappearance of pyruvate can be observed. This is particularly marked when 2 mg. or more of pyruvate is injected per cornea. With such a large surplus of substrate the rate of consumption is approximately 400 micrograms per hour per cornea. This is to be compared with the previously reported rates of consumption of glucose (100 micrograms per hour) and of glycogen and lactate (each 25 micrograms per hour). With lower concentrations of pyruvate in the tissue the rate of disappearance of pyruvate falls off roughly in proportion to the concentration. Since the pyruvate is consumed almost exclusively by the epithelium, one may compare the activity of this tissue with some others for which data are available in the literature. The wet weight of the epithelium of one cornea is approximately 80 mg. Since this is capable of consuming 400 micrograms per hour we reach a figure of about 5×10^{-2} mM per gram wet weight per hour. This is comparable to the rate found in heart muscle by D. H. Smyth (5).

The rate of pyruvate disappearance in these experiments is uninfluenced by anaerobiosis (Table II) or by exposure of the tissue to mustard (Table III), iodoacetate, fluoride, or arsenite (Table IV). The possible effect of mustard was tested for by injecting pyruvate into the cornea not only immediately after exposure to mustard but also after incubation periods of 4–8 hours following exposure. This was done in order to disclose the possibly delayed appearance of a mustard effect, but the consumption of pyruvate was normal in all these experiments.

2). *Lactate Production.* The fact that the rate of pyruvate disappearance was the same aerobically and anaerobically, the further fact that the aerobic disappearance of pyruvate was not associated with any increase in the oxygen uptake, both indicated that we were dealing with a dismutation rather than an oxidation of pyruvate. This conclusion was confirmed by the finding that approximately one half of the pyruvate which disappears can be recovered as lactate

TABLE II

Pyruvate Disappearance and Lactate Production under Anaerobic Conditions

Incubation time: 4 hours.

Temperature: 31°C.

	PYRUVATE ADDED	MICROGRAMS PER CORNEA					ESTI- MATED PYRUVATE CON- SUMED	ESTI- MATED LACTATE PRODUCED FROM PYRUVATE
		Pyruvate		Lactate				
Before incubation.....	+	540	500	385	420	630	360	
Incubated under H ₂	—			570	620			
Incubated aerobically.....	+	150	170	570	535			
Incubated in evacuated Thunberg tubes.....	+	150	130	880	890		380	280
Incubated under H ₂	+	100	120	785	760		410	170

TABLE III

*Disappearance of Pyruvate from Beef Cornea after Exposure to Mustard Vapor for 15 Minutes*Estimated uptake 0.6 micrograms mustard per cm² per minute.

Amount of pyruvate used for injection: 1 mg.

Corneas per sample: 2.

Incubation Temperature: 28°C.

Controls injected with pyruvate and incubated but not exposed to mustard.

INCUBATION TIME BETWEEN EXPOSURE TO MUSTARD AND INJECTION OF PYRUVATE	INCUBATION TIME AFTER INJECTION OF PYRUVATE	MICROGRAMS OF PYRUVATE RECOVERED PER CORNEA			
	None	Control	540	560	
4 hours	5 hours	Control	100	90	
		Treated	97	95	
12 hours	5 hours	Control	60	60	
		Treated	80	90	
5 hours	5 hours	Control	170	130	130
		Treated	170	160	145
8 hours	2 hours	Control	300	370	350
		Treated	350	360	310

(Table V). The rate of reaction as measured by the lactate production was also uninfluenced by anaerobiosis (Table II) or by exposure to mustard.

has no measurable oxygen uptake, should fail to oxidize pyruvate, but it is rather remarkable that only small losses of pyruvate occur by dismutation or reduction.

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		Pyruvate		Lactate				
Before incubation.....	+	540	500	385	420	630	360	
Incubated under H ₂	-			570	620			
Incubated aerobically.....	+	150	170	570	535			
Incubated in evacuated Thunberg tubes.....	+	150	130	880	890		380	280
Incubated under H ₂	+	100	120	785	760		410	170

TABLE III

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Amount of pyruvate used for injection: 1 mg.

Corneas per sample: 2.

Incubation Temperature: 28°C.

Controls injected with pyruvate and incubated but not exposed to mustard.

INCUBATION TIME BETWEEN EXPOSURE TO MUSTARD AND INJECTION OF PYRUVATE	INCUBATION TIME AFTER INJECTION OF PYRUVATE	MICROGRAMS OF PYRUVATE RECOVERED PER CORNEA			
	None	Control	540	560	
4 hours	5 hours	Control	100	90	
		Treated	97	95	
12 hours	5 hours	Control	60	60	
		Treated	80	90	
5 hours	5 hours	Control	170	130	130
		Treated	170	160	145
8 hours	2 hours	Control	300	370	350
		Treated	350	360	310

(Table V). The rate of reaction as measured by the lactate production was also uninfluenced by anaerobiosis (Table II) or by exposure to mustard.

TABLE IV

Effect of Metabolic Inhibitors on Pyruvate Utilization

Incubation time: 4 hours.

	PYRUVATE CONTENT IN MICROGRAMS PER CORNEA	
Before incubation.....	1330	1330
Controls.....	630	500
Sodium arsenite, 0.01 M.....	700	610
0.02 M.....	790	900
Iodoacetate, 0.01 M.....	650	680
Before incubation.....	610	620
Controls.....	240	250
Fluoride, 0.02 M.....	240	180
Malonate, 0.025 M.....	240	180

TABLE V

Pyruvate Disappearance and Lactate Production

Amount of pyruvate used for injection: 2 mg. per cornea.

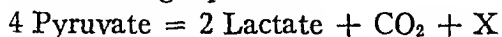
Incubation temperature: 29°C.

		PYRUVATE MICROGRAMS PER CORNEA	LACTATE MICROGRAMS PER CORNEA	ESTIMATED PYRUVATE CONSUMED MICROGM. PER CORNEA	ESTIMATED LACTATE PRODUCED FROM PYRUVATE MICROGM. PER CORNEA
Immediately after injection	No substrate added		315, 290, 350		
	Pyruvate in- jected	1240, 1330	250, 285 265, 275, 270 275, 285		
Incubation time 2 hours	No substrate added		345, 320		
	Pyruvate in- jected	850, 1020	405, 425	350	117
4 hours	No substrate added		195, 220, 200 200, 190		
	Pyruvate in- jected	560, 530	505, 600, 440 430, 425	750	300

It must be remembered that our experiments were performed on whole corneas with their reserves of endogenous substrates intact. Under these circumstances the endogenous substrates compete for the

oxidation available to them through the hydrogen transport system. This hydrogen transport system appears to be operating at almost its maximum capacity in relation to the endogenous substrates, and not to be available for the very large amount of pyruvate which we supplied. Consequently, our finding that under the conditions of our experiment pyruvate disappears by dismutation, rather than by oxidation, does not imply that pyruvate is consumed in this tissue only by dismutative processes. On the contrary, the fact that the total O_2 uptake under normal circumstances closely matches the total carbohydrate consumption strongly suggests that pyruvate oxidation occurs in this tissue. In some preliminary experiments in which we have used corneal epithelium instead of whole corneas, an increase in O_2 uptake was found in the presence of pyruvate. It may well be that the partial inhibition after exposure to mustard, which we found in the consumption by the epithelium of its endogenous lactate (as distinguished from its consumption of stroma lactate), may be the result of an inhibition of pyruvate oxidase by mustard similar to that noted by Peters and Wakelin (6) in other tissues.

3) *CO₂ Production.* CO_2 was measured manometrically both aerobically and anaerobically. In each case the difference between the gas produced in the presence of pyruvate and in its absence amounted to about one mole CO_2 produced for each 4 moles pyruvate consumed. The general reaction can, therefore, be expressed approximately in the following equation:



The aerobic experiment is shown in Table VI. Corneas were placed in bicarbonate buffer with air in the gas phase. In the absence of pyruvate the gas phase contracts on incubation (O_2 consumption minus CO_2 production). At the end of the incubation period HCl solution in the side arm is tipped into the main chamber liberating all the bicarbonate as CO_2 . The difference in the total change in volume to the end of the experiment after addition of HCl, between controls and pyruvate containing vessels, is taken as the measure of the CO_2 produced from pyruvate. It is interesting that precisely the same difference in volume change is noted at the end of the incubation period, and that the same amount of CO_2 is liberated from the bicarbonate solution on addition of HCl in all samples. This can only

mean that in the dismutation of the pyruvate there is no change in the total number of fixed acid groups while one excess acid equivalent appears as CO_2 . It follows that X in the equation includes two monobasic acid groups. If one were to take the above formula as exact, which probably it is not, the molecular formula of X would be equivalent to that of alpha-keto glutaric acid.

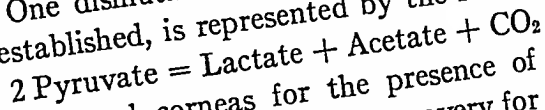
4) *Acetaldehyde and other Aldehydes*. No acetaldehyde could be recovered from control corneas and none accumulated during the consumption of large amounts of pyruvate. Tests for reducing sugars

TABLE VI
CO₂ and Lactate Production by Beef Corneas Incubated with and without Added Pyruvate

	CHANGE OF GAS VOL. IN CU.MM.	CHANGE OF GAS VOL. IN CU.MM. ON ADD. OF HCl		CHANGE OF LACTATE CONTENT IN MICROGRAMS	CHANGE OF PYRUVATE CONTENT IN MICROGRAMS
		Before Incubation	After Incubation		
No substrate added	-53 -49	+72	+80 +87	-60 -50	
2 mg. pyruvate added per cornea	+2 +1	+84	+90 +98	+260 +310	-720 -700
ESTIMATED PYRUVATE UTILIZED	ESTIMATED LACTATE PRODUCED FROM PYRUVATE		ESTIMATED PYRUVATE USED TO YIELD CO_2		
			207		
710	330				

and for glycogen showed no differences between controls and pyruvate consuming samples.

5) *Acetate*. One dismutative reaction, the possible occurrence of which is well established, is represented by the following formula:



Accordingly, we tested corneas for the presence of acetate. The method described above gave satisfactory recovery for a pure solution of acetate in amounts such as we had to expect in the corneal extracts. Additions of pyruvate and of lactate to the solution of acetate did not interfere with the recovery. The cornea itself does not contain significant amounts of volatile acids (Table VII), nor could any increase of volatile acid be detected on incubation for four hours at 29°C .

after injection of pyruvate, in spite of the fact that previous experiments showed that the greater part of the injected pyruvic acid disappears within this time. If only one tenth of the pyruvic acid which was injected into three corneas would have been transformed into

TABLE VII

Determinations of Acetate in Pure Solutions and in Corneal Extracts

	ml. .01 N NaOH
Acetic acid solution 1 ml.....	3.07
Recovery on distillation.....	2.78, 2.92
	2.80, 2.85
Acetic acid solution 0.5 ml.....	1.60
Recovery on distillation.....	1.52
Acetic acid solution 1 ml.....	2.72
Recovery on distillation.....	2.40
Recovery on distillation with 2 mg. Pyruvic acid added.....	2.59
8 mg. Lactic acid added.....	2.62
15 mg. Lactic acid added.....	2.55
<i>Three Corneas per Samples</i>	
Corneal extract.....	0.25, 0.34
Extract after injection of 4 mg. pyruvic acid per cornea.....	0.41, 0.38
Extract from cornea injected with 4 mg. pyruvate per cornea and incubation for 4 hours at 28°C.....	0.44, 0.32
Same extract after addition of acetic acid equivalent to 1.45 ml. .01 N NaOH....	1.43
Recovery of acetate after injection into corneas immediately after injection....	1.67, 1.90
	1.52, 1.62
	1.82, 1.98
	1.78
Average.....	1.74, 1.67
after incubation for hours at 28°C.....	1.37, 1.63
	1.45
Average.....	1.57

acetic acid, titration values of about 1 ml. of .01 N NaOH should have been obtained. In order to exclude the occurrence of substances which might interfere with the distillation of acetic acid, an arbitrary amount of acetic acid was added to the extract of the incubated sample. About 70% of the added acetate could be recovered.

The absence of acetate after incubation of corneas with pyruvate could also be demonstrated with the lanthanum reagent of Krüger and Tschirch (7). Extracts from control corneas to which acetate was added gave clear positive reactions. Extracts from corneas which were incubated for four hours after injection of pyruvate gave negative results with the same reagent. From this series of experiments the formation of acetate from pyruvate seemed unlikely. However, we had still to exclude the possibility that acetate is utilized as rapidly as it is formed from pyruvate and therefore escapes detection. The rate of its disappearance was determined by comparing the amount recoverable immediately after injection with the amount recoverable after 4 hours of incubation. From the results listed in Table I, it can be seen that only a very small fraction of the injected acetate disappears, and if acetate would be formed in the course of the rapid disappearance of pyruvate it should accumulate in readily detectable amounts. The rate of consumption of acetate was 15 micrograms per cornea per hour.

6) *Acetoin*. Since acetate apparently is not formed from pyruvate, possibilities other than dismutation were examined. In the following experiments we investigated the formation and disappearance of acetoin. The characteristics common to the observed pyruvate disappearance in the cornea and the condensation of pyruvate to acetoin are the independence of O_2 supply, the production of CO_2 , and the relative insensitivity to arsenite (8). On the other hand we had to postulate, in this case, an independent reaction in order to account for the appearance of lactic acid. After injection of pyruvate in amounts from two to four mg. per cornea and an incubation for 2-4 hours, no acetoin could be detected although acetoin, added to the same corneal extracts, could be recovered with a yield of 80%. However, acetoin itself disappears at a rapid rate from the cornea. After injection of 2 mg. per cornea about 0.4 mg. disappeared in three and a half hours, and after injection of 1 mg., per cornea 0.25 mg. was utilized in the same period (Table VIII). If it is taken into account that the formation of one molecule of acetoin (mol. weight 88) requires the condensation of two molecules of pyruvate (mol. weight 87), the higher value would correspond to a consumption of 800 micrograms of pyruvate.

This figure approaches our experimental data for the maximum pyruvate utilization (1000–1200 microgm. per cornea) and would indicate that acetoin disappears from the cornea almost as fast as pyruvate. Therefore, at the present time, acetoin formation cannot be excluded with certainty as a pathway of pyruvate utilization in the cornea, although no data are available as yet to provide positive evidence for this possibility.

TABLE VIII
Utilization of Acetoin

Incubation time: 4 hours.

Temperature: 29°C.

AMOUNT OF ACETOIN INJECTED MICRO- GRAMS PER CORNEA	AMOUNTS OF ACETOIN RECOVERED IN MICROGM. PER CORNEA				
	Before incubation	After incubation			
		Controls		Exposed for 15 min. to mustard vapor	
		recovered	utilized	recovered	utilized
2000	1250	900	350	750	500
	1200	900	300	700	500
	1350	850	500	750	600
	1400	1050	350	850	550
		950	450	800	600
	1400	1000	400	900	500
	1300	1050	250	1050	250
		1050	250		
1000	800	600	200	450	350
	900	650	250	550	350
Average.....			330		477

It was noted above that the disappearance of pyruvate is not inhibited by exposure to mustard. It was of interest, therefore, to determine whether the utilization of acetoin would prove equally insensitive to the application of mustard. The eyes were exposed to mustard vapor and then kept three and one half hours at room temperature in order to provide sufficient time for the reaction of mustard with the cell constituents. After this preliminary period the acetoin was injected and the corneas excised and incubated for 4 hours at 29°C.

From the results in Table VIII it can be seen that exposure to mustard does not inhibit the disappearance of acetoin. On the contrary, a slightly accelerated disappearance is indicated in the exposed corneas.

7) *Butyrate*. The tests on the utilization of butyric acid were carried out in order to obtain some information about the rate of

TABLE IX

Utilization of Butyrate

Butyrate used for injection: 500 micrograms per cornea.

Incubation temperature: 29°C.

INCUBATION TIME	RECOVERY OF BUTYRIC ACID IN MICROGM. PER CORNEA		
	Before incubation	Incubated controls	Incubated after exposure to mustard
10 hours	510	230	
	440	430	
	520	280	
		340	
20 hours	580	40	
	330	10	
		50	
		50	
	430	180	240
	290	50	210
	300		300
	360		240
	500	190	290
	480	250	260
	400	20	200
	390	50	80
	390	100	200
	290	170	
Average.....	400	100	220
Utilized.....		300	180

utilization of fatty acids in the cornea and to examine, if possible, the effect of exposure to mustard on the metabolism of a fatty acid; the determination of which is technically less disagreeable than the determination of acetic acid. The metabolism of butyrate is of additional interest since it is the metabolic precursor of the ketobodies

(acetoacetic acid, hydroxybutyric acid) which are metabolically very reactive substances. A possible connection with the preceding experiments arises out of the fact that acetoacetic acid is the product of one of the condensation reactions of pyruvate.

For the determination of butyric acid we used the procedure described above. The utilization of butyrate amounts to about 400 micrograms per cornea in 20 hours. This metabolic process is slow compared with pyruvate or acetoin utilization and about equal to the rate of consumption of lactate. Exposure to mustard has a marked inhibitory effect on the utilization of butyrate (Table IX). The question as to whether the utilization of butyrate is limited to the corneal epithelium as in the case of lactic acid has not been tested.

SUMMARY

The denuded corneal stroma consumes pyruvate at a very low rate, but when the epithelium is present pyruvate injected into the stroma is consumed at an extremely rapid rate. The consumption of pyruvate, in experiments on whole cornea, is uninfluenced by anaerobiosis or by exposure to mustard, iodoacetate, fluoride, or arsenite. About half of the pyruvate lost can be recovered as lactate, indicating that the process is at least in part a dismutation. For each 4 moles of pyruvate lost approximately 1 mole of CO_2 is recovered. The remainder is as yet unaccounted for. It may be ketoglutarate. It is not acetate, or acetaldehyde, or reducing sugar, or glycogen. Acetoin does not accumulate in the cornea during pyruvate consumption, but acetoin itself is consumed by the cornea at almost the same rate as pyruvate and its consumption is not inhibited by mustard. It cannot, therefore, be excluded as a possible product of pyruvate consumption. The consumption of butyrate was tested for comparison and found to be slow relative to pyruvate. Its consumption is inhibited by exposure to mustard.

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XIV. THE UTILIZATION OF RIBOSE AND OTHER PENTOSE BY THE CORNEA*

HEINZ HERRMANN AND FAY H. HICKMAN

The occurrence of ribose in nucleotides which act as the prosthetic group of enzymes, in coenzymes, and in nucleic acid, gives special importance to this substance, but information on the metabolic fate of ribose and other pentoses in animal tissues has advanced extremely slowly. The possibility of pentose formation from hexuronic acids, and of the condensation from triose and a C_2 precursor are discussed in recent reviews (1). The existence of a metabolic relation between uronic acids and pentoses is indicated by the finding that pentoses are excreted in the urine in higher concentrations in individuals producing excessive amounts of glucuronic acid, for instance, in the course of detoxification reactions (2). Lipmann (3) and Warburg (4) touched the problem of pentose metabolism in their investigations on the oxidation of phosphohexonic acid. The amount of oxygen consumed indicated that pentose was formed and that even 4 carbon and 3 carbon chains might appear as breakdown products. It should be recalled, however, that not ribose but arabinose is to be expected from the oxidation of the physiological hexoses. An inversion of one carbon atom in the hexose molecule would be required to yield ribose as an oxidation product. Dickens (5) studied the oxidation of pentose phosphates by Lebedew juice and also by animal tissues, and demonstrated the oxidation of ribose phosphate by red blood cells. Dische (6), who studied the metabolism of adenosine by laked red blood cells, obtained evidence suggesting the breakdown of the ribose part of the molecule into triosephosphate and a two carbon compound, possibly glycolaldehyde. More recent publications deal with attempts at elucidating the mechanism by which ribose is utilized for the synthesis of nucleotides (7). In the present paper we studied the disappearance of the ribose from the excised surviving cornea and tested the effect of various metabolic inhibitors and of mustard on this process.

* The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

EXPERIMENTS

1. *Methods.* The experiments were performed on beef corneas. The technique of maintenance of the corneas and of the injection of various substrates and inhibitors was the same as that described in previous papers. When ready for the chemical analysis, the tissue was ground in trichloroacetic acid, centrifuged, and the supernatant fluid used for ribose determination.

Mejbaum's (8) modification of the Bial test was used to estimate pentose plus pentose nucleotide in the tissue extracts, the colorimetric reading being made with a Klett Summerson photoelectric colorimeter used with a #66 filter. Pentose standards containing 25 and 50 micrograms of ribose were carried along with each set of experiments. Glucose, fructose, ascorbic acid, and glucosamine interfere to such a slight extent with this colorimetric test under the prescribed conditions that their influence on the reading could be neglected. Glucuronic acid, however, produces a color of almost the same intensity as that which is obtained with pentose, since under the influence of hot, strong HCl as used in this test decarboxylation of glucuronic acid and formation of pentose may take place.

Estimates of injected ribose were also made with the reduction method (9) previously used for glucose determination. Standards and blanks were run along in all determinations.¹

2. *Results.* Some material is extractable from the cornea which gives a positive Bial test. The amount is equivalent to about 200 micrograms ribose per cornea. One-third of this amount can be accounted for as adenosine triphosphate, and the rest is probably derived from other nucleotides. The greater part of this material is found in the epithelium (Table I). Since the corneal mucoïd is confined to the stroma, the small stroma fraction in the Bial titration indicates that the interference of glucuronic acid in the determination is at most very small.

d-Ribose injected into the cornea disappears at an appreciable rate. In the experiments shown in Table II, 0.25 ml. containing 1 mg. d-ribose

¹ The pentoses used in this study were obtained from the following sources: d-Ribose, d-Xylose, and d-Lyxose were obtained from Pfanstiehl Chemical Co. Waukegan, Ill., and the d-Arabinose was obtained from Eastman Kodak Company, Chemical Division, Rochester, N. Y.

was injected into each beef cornea. About one-half of the injected fluid runs out of the needle holes immediately after the injection. This immediate mechanical loss is unavoidable in our experiments but by careful control of the injection technique can be kept reasonably uniform. The metabolic experiment, therefore, begins with 600—700 micrograms per cornea of which 200 is endogenous. After an incubation period of 20 hours at 32°C, the injected material has almost completely disappeared. The rate of disappearance of d-xylose and d-

TABLE I
Pentose (Bial Test) in Beef Cornea Calculated as Ribose

	MICROGRAMS PER CORNEA
Whole Cornea.....	163, 156, 214, 207
Epithelium.....	170, 176, 180, 174
Stroma.....	40, 23, 33, 44

TABLE II
Utilization of Injected Pentose by Beef Cornea during Incubation for 20 Hours at 32°C.
1 mg. injected.

MICROGRAMS PER CORNEA							
d-Ribose		d-Xylose		d-Arabinose		d-Lyxose	
Before	After	Before	After	Before	After	Before	After
Incubation		Incubation		Incubation		Incubation	
734	360	651	309	660	615	705	510
582	222		423	654	519		495
603	300		313	564	525		556
726	320		383	630			593
673	336		296	531			

ribose is much greater than that of d-lyxose and d-arabinose. This difference may be due either to a difference in the actual rate of metabolic breakdown or to a difference in the rates with which the various pentoses penetrate into the epithelium.

The corneas into which ribose has been injected preserve their stores of glycogen (Table III) in the same way as do corneas injected with glucose solution. There is also a marked inhibition of lactate disappearance (Table IV).

The rate of disappearance of ribose in the presence of various metabolic inhibitors was examined. Iodoacetate exerts a marked inhibitory effect. The threshold for iodoacetate inhibition of glucose utilization is, however, still lower. Thus with 0.3 mg. of iodoacetate per cornea

TABLE III

Effect of Injection of Ribose and Glucose on the Glycogen Content of the Cornea after Incubation for 20 Hours at 32°C.

1 mg. sugars injected.

CONTROL	GLYCOGEN CONTENT IN MICROGRAMS PER CORNEA	
	Ribose	Glucose
600	908	933
575	756	1075
	1002	

TABLE IV

Effect of Injection of Ribose on the Lactic Acid Content of the Cornea after Incubation for 12 Hours at 32°C.

1 mg. Ribose injected.

LACTIC ACID CONTENT IN MICROGRAMS PER CORNEA	
Without Ribose	With Ribose injected
123	273
140	296
113	273
80	250
116	280
130	213
60	160
26	236
Average.....99	247

(about 2×10^{-3} M), glucose utilization is diminished by 70% but ribose utilization by only 30% (Table V).

In table V experiments are listed in which ribose disappearance was followed both by the Bial test and by ferricyanide reduction. The effect of iodoacetate in inhibiting the utilization of ribose is apparently stronger in respect to reducing power of the injected substrate than in

respect to the Bial test. Thus, by the application of iodoacetate, two steps in the metabolic breakdown of ribose become discernible. The first step results in the formation of a product which fails to give the Bial reaction but still has reducing capacity. In the second step re-

TABLE V

Comparison of the Effect of Iodoacetate on the Utilization of Ribose and Glucose

	MICROGRAMS OF SUGAR PER CORNEA				
	Before Incubation	After Incubation 20 Hours at 32C°			
IODOACETATE INJECTED.....	0	0	0.3 mg.	0.5 mg.	1 mg.
Ribose Bial Test	700	294 276	396	430 400	438
	699	270 294	350	386 566	523
	783	353 282	248	366 565	570
	736	380 303	403	666 438	456
	889	290 260	423	626	566
	696	316 313 320		366	
Average.....	751	304	364	480	511
Ribose Ferricyanide Test	823	340 266	386	576 623	666
	873	326 262	466	546 611	716
	733	335 320	473	513 654	713
	693	226 300	466	693 726	
Average.....	781	297	448	618	698
Glucose Ferricyanide Test	853	285	678	720	
	800	280	613	766	
	826	262	604		
	853	205	706		
		306 326			
Average.....	833	277	650	743	

ducing capacity is lost. Iodoacetate apparently inhibits the second step more strongly than the first. Since even the disappearance of reducing capacity is less sensitive to iodoacetate inhibition in the case of ribose than in the case of glucose, it would seem improbable that the reducing substance derived from ribose is triosephosphate. Dische

(6) has described a breakdown product of ribose which has reducing power, and it may be that this is the intermediate which is formed in the

TABLE VI

Effect of Various Metabolic Inhibitors on the Utilization of Ribose

1 mg. Ribose injected.

AMOUNT INHIBITOR INJECTED PER CORNEA		MICROGRAMS OF RIBOSE PER CORNEA DETERMINED AFTER INCUBATION FOR 20 HOURS AT 32°C.					
		Inhibitor Added			Control		
Fluoride	1.0 mg.	633	573	603	290	333	
	0.3 mg.	383	303	336	326		
	0.1 mg.	300	350	193	233		
Arsenite	1.0 mg.	360	350	373	193	270	193
	0.5 mg.	273	285		276	210	
Malonate	1.0 mg.	290	243	320	293	250	276

TABLE VII

Effect of Treatment with Mustard on the Utilization of Ribose by the Cornea during Incubation for 20 Hours at 32°C.

1 mg. Ribose injected.

EXPOSURE TIME IN MINUTES	MICROGRAMS RIBOSE PER CORNEA	
	Control	Treated
10	249	261
	203	243
	210	282
	240	276
20	207	369
	213	295
	240	345
30	390	483
	346	490
	270	343
	286	362

cornea. Of the other metabolic inhibitors, tested fluoride gives a marked inhibition of ribose utilization, arsenite has a relatively small effect, and malonate is without influence (Table VI).

The effect of exposure of the cornea to mustard on the subsequent utilization of ribose is very small (Table VII). Corneas which had been exposed to mustard vapor at room temperature for 10 minutes² showed a normal rate of disappearance of ribose. Exposure for 20—30 minutes resulted in a slight decrease in the rate of disappearance of ribose.

In the small inhibition of ribose utilization which was observed after prolonged exposure to mustard vapor, no discrepancy was found between the results of the Bial test and of ferricyanide reduction, indicating that mustard does not preferentially inhibit the second step in the breakdown as does iodoacetate.

DISCUSSION AND SUMMARY

Our experiments show a slow but steady utilization of d-ribose and d-xylose by corneal tissue. The rate of disappearance is about 10—12 micrograms per hour per cornea, which is about one-tenth that of the rate of utilization of glucose and one-half that of lactate and glycogen.

d-Lyxose is utilized only at half the rate of d-ribose, and with d-arabinose no disappearance could be measured with certainty. High selectivity of cells in respect to utilization of pentoses has become known in case of the absorption of pentoses from intestine (10) and the metabolic utilization by various bacteria (11). In our case it remains undecided as to whether the observed difference in the utilization of pentoses by the cornea rests in the specificity of the permeability or in the specificity of the effective enzymes.

In the pathway of ribose utilization two steps became discernible by the use of iodoacetate. As pointed out above, at a proper concentration of this metabolic inhibitor, the first step in the ribose breakdown takes place at a slightly diminished rate only, while a subsequent reaction is greatly inhibited, leading to an accumulation of a breakdown product with reducing properties. During an incubation for 20 hours, an amount of this intermediary accumulated which corresponds to 150 micrograms of ribose per cornea. With experiments on a larger scale it should be possible to obtain sufficient material to identify this intermediary.

² Under the conditions of these experiments the estimated uptake of mustard was 0.6 micrograms per cm² per minute.

We have no indication as yet as to whether the breakdown of ribose in the cornea involves phosphorylation. The fact that the utilization of ribose has been observed with highly viable and intact cells, and yet under conditions which allow complete analysis, gives some promise that syntheses with ribose, e.g. nucleotide formation, could be observed with this tissue.

The utilization of ribose is markedly inhibited by iodoacetate and fluoride, but is only slightly inhibited by mustard. In the presence of ribose the utilization of glycogen and of the lactate depot in the cornea is suppressed.

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XV. STUDIES ON NON-PROTEIN NITROGEN IN THE CORNEA*

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The present studies were undertaken in preparation for the more ambitious project of investigating the nitrogen metabolism of the cornea with the aid of isotopically labelled compounds. When the work had to be abandoned on account of technical difficulties, it was still found possible to study the production and utilization of some nitrogenous substances in the cornea for which suitable microanalytic methods were available.

TECHNIQUE

The experiments were performed on beef corneas. The preparation, exposure to mustard,¹ and incubation of corneas have been described in previous papers. Only uninjured and perfectly clear corneas were used in these experiments. Corneas were selected carefully with regard to uniformity of size in each experiment. After incubation the corneas were vigorously ground for 10 minutes, with addition of sand, in 5% trichloroacetic acid, two corneas being used for each sample. The extract was poured into a filter and the extraction repeated twice with 3 ml. and 2 ml. of trichloroacetic acid solution, grinding the tissue each time for two minutes. The combined filtered extracts were made up to 10 ml., one half of which was used for ammonia determination, the other half for NPN determination. The portions used for ammonia determination were alkalinized with one drop of 40% NaOH and 2.5 ml. of a saturated solution of potassium carbonate. A gentle stream of air carried the ammonia into a receiver containing 5 ml. of half saturated boric acid to which brom-cresyl-green was added as indicator. The ammonia was then titrated with N/100 H₂SO₄. The results are tabulated below as ammonia, but in work with tissues it should be

* The work described in this paper was done in part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

¹ Under the conditions of exposure used in these experiments it was estimated that 0.6 micrograms of mustard were taken up per cm² per cornea per minute.

kept in mind that by this method volatile amines are determined as well.

Determinations of amino nitrogen were carried out according to the method of Peters and van Slyke (1). In preparing the extracts in this series of determinations we avoided the tedious manual grinding of the tough corneal tissue by using the mechanical extractor which has been described elsewhere (2). The trichloroacetic acid extracts of the corneas obtained with this instrument were filtered and evaporated to dryness in order to remove the trichloroacetic acid. The residue was dissolved in 10 ml. of distilled water and a 5 ml. portion was used for amino nitrogen determinations. The second portion was used for determination of total NPN by microkjeldahl.

For the determination of serine, three corneas were pooled for each sample when whole corneas were examined, and 6 to 9 corneas for the determinations on separated epithelium or stroma. Those corneas injected with dl serine received 2.0 mg. in 0.25 ml. of 0.3% saline. Unless otherwise indicated, the corneas were incubated at 38°C for approximately 10 hours. The tissues were extracted in 5% trichloroacetic acid, the extracts were neutralized, and serine determinations performed on the whole extract according to the procedure of Boyd and Logan (3) as follows: The samples of approximately 15 ml. were transferred to a 300 ml. Kjeldahl flask and 3 drops of methyl red and 4 ml. of 25% potassium arsenite added. Approximately 3.0 ml. of 0.5 M periodic acid was required to adjust the reaction mixture to the proper pH. The formaldehyde was distilled off and determined colorimetrically. For the analysis of samples containing less than 1.0 mg. serine, 2.5 ml. aliquots of the distillate were put into test tubes calibrated at 25 ml., 0.25 ml. of the purified chromotropic acid reagent were added, and the volume made up to 8.5 ml. with distilled water. The tubes were cooled in an ice bath and 5.0 ml. of concentrated sulfuric acid added slowly with constant shaking. The mixtures were allowed to cool to the temperature of the ice bath and then made up to 25 ml. with concentrated sulfuric acid. They were placed in a boiling water bath for ten minutes, cooled and read with a #56 filter in a Klett Summerson photoelectric colorimeter.

The specificity of the test is not absolute. It rests upon the oxidation of serine to formaldehyde by periodic acid and the subsequent

colorimetric determination of the formaldehyde. The reaction for formaldehyde is quite specific, but there are potentially in the tissue some substances, other than serine, which will yield formaldehyde on oxidation with periodic acid, and hence will be titrated as serine. Determinations of endogenous serine by this method are, therefore, somewhat equivocal. We undertook measurements on the endogenous serine, or more properly the endogenous formaldehyde producing substances, essentially as controls for subsequent experiments in which much larger amounts of serine were injected into the tissue. In these experiments the small fraction of endogenous formaldehyde producing substances was negligible.

(1) *Non-Protein Nitrogen*. The determination of the non-protein nitrogen (NPN) level in the excised cornea was carried out for several reasons. The data were needed as a basis of experiments on utilization of externally supplied nitrogen, which made it necessary to find out whether during incubation the level in the whole cornea and in the stroma would remain constant or would show a definite change. Possible small changes in the NPN level seemed of interest also in connection with the mechanism of the loss of adhesion of the epithelium. We observed that among the enzymes tested only trypsin and chymotrypsin caused a loosening of the corneal epithelium and a similar observation was made by Medawar (4) on skin. Therefore, it seemed possible that proteolytic processes, detectable by changes of the NPN level, might play a role in the loosening. In the case of vesication caused by thermal burns (5) and by mustard (6), some connection with proteolytic processes has been suggested by Peters and his coworkers.

Results. The normal beef cornea contains about 250 micrograms of extractable non-protein nitrogen, of which slightly less than 10% is recovered as ammonia. About half of the total nitrogen and about 80% of the ammonia is in the stroma. Since the wet weight of the epithelium is about one tenth that of the stroma, the concentration of NPN in the epithelium is about 5 times that in the stroma, while the concentration of ammonia in the two tissues is more nearly equal.

On incubation either of the whole cornea or of the isolated stroma, the ammonia content remains fairly constant. This is true both for controls and for tissues previously exposed to mustard.

On incubation either of the whole cornea or of the isolated stroma,

only very small changes were observed in the NPN content in normal controls, and the direction of the change was inconsistent. If, however, the whole cornea was incubated for 3 hours or more after exposure

TABLE I

Ammonia and Total Non-Protein-Nitrogen Content of the Whole Cornea and of the Isolated Stroma after Exposure to Mustard

Exposure time to Mustard: 15 minutes.

Incubation: 9½–10 hours at 28–32°C.

MICROGRAMS NITROGEN PER CORNEA					
Ammonia Nitrogen			Total Extractable Non-Protein-Nitrogen		
Before Incubation	After Incubation		Before Incubation	After Incubation	
Control	Control	Exposed to Mustard	Control	Control	Exposed to Mustard
Whole Cornea					
20.8	21.5	20.5	249.5	235.5	323.0
15.8	14.3	13.6	229.7	248.5	274.2
13.6	12.9	15.8	240.0	228.0	304.0
15.2	20.0	21.5	196.3	223.9	277.0
18.4	21.5	17.9	264.8	231.8	290.3
			270.0	226.3	254.5
			272.3	284.7	337.8
			255.0	259.3	303.0
			229.9	251.0	289.8
			183.0	203.0	272.8
			272.0	257.0	301.0
Av.....16.7	18.0	17.8	242.1	240.8	292.5
Isolated Stroma					
18.0	14.3	15.1	132.8	130.4	138.0
12.0	11.6	11.6	128.0	135.0	125.0
14.1	12.9	19.3	134.2	123.8	136.4
14.4	12.9	14.4	123.6	107.5	106.0
Av.....14.6	12.9	15.1	129.7	125.1	126.4

to mustard there was a regular increase in NPN of 10–20%. This increase after exposure to mustard was not observed if only the isolated stroma was incubated (Table I).

After exposure to mustard and incubation of whole corneas for two hours no increased NPN was observed, but after three hours of incu-

bation the greater part of the excess NPN was already present. Some additional increase was found on incubation up to 10 hours,

TABLE II

Total Non-Protein-Nitrogen after Varying Lengths of Incubation

Exposure time to Mustard: 15 minutes.

Incubation temperature: 28°C.

MICROGRAMS NITROGEN PER CORNEA				
Incubation Time	Before Incubation	After Incubation		
	Control	A Control	B Exposed to Mustard	Difference B-A
2 hrs.		262.0	261.0	-1
		257.5	261.1	+3.5
3 hrs.		249.5	282.0	32.5
		246.5	281.5	25.0
4 hrs.	250.0	254.5	264.5	10.0
		268.0	279.0	11.0
		255.0	299.0	44.0
		261.0	287.0	26.0
5 hrs.		251.0	283.0	32.0
		264.5	284.0	19.5
6 hrs.	265.3	272.5	314.7	42.2
	250.0	258.0	301.5	43.5
		262.0	292.8	30.8
10 hrs.		259.3	303.0	43.7
		245.5	313.0	67.5
		260.5	318.0	57.5
		236.3	290.5	54.2
		263.0	307.0	44.0
		284.7	337.8	53.1
20 hrs.	224.0	217.0	276.0	59.0
		247.0	304.0	57.0
		256.0	324.0	68.0
		243.0	323.0	87.5

but prolonged incubation up to 20 hours yielded but a very slight increase in NPN (Table II).

The full effect in respect to NPN increase is produced by 15 minutes

exposure to mustard vapor at room temperature. Increase of the exposure time up to 90 minutes yielded no greater excess of NPN. When the exposure time was reduced to 5 minutes the yield of increased NPN was reduced to about half (Table III).

TABLE III
Total Non-Protein-Nitrogen after Varying Lengths of Exposure to Mustard
Incubation: 10 hours at 28°C.

MICROGRAMS NITROGEN PER CORNEA					
Length of Exposure	Before Incubation Control	After Incubation			
		A Control	B Exposed to Mustard	Difference B-A	Average Difference
5 min.		263.0	277.0	14.0	26.7
		236.3	258.0	21.7	
		260.5	282.0	21.5	
		245.5	295.0	49.5	
10 min.		236.3	274.0	38.0	35.2
		263.0	285.5	22.5	
		268.0	313.0	45.0	
15 min.		259.3	303.0	43.7	53.3
		245.5	313.0	67.5	
		260.5	318.0	57.5	
		236.3	290.5	54.2	
	263.0	307.0	44.0		
	284.7	337.8	53.1		
60 min.	275.3	284.7	339.0	54.3	37.8
	255.0	259.3	307.3	48.0	
	229.8	251.0	272.0	21.0	
90 min.	183.0	203.0	248.0	45.0	43.0
	275.0	257.0	310.0	53.0	
		268.0	299.0	31.0	

When the temperature of incubation was varied between 28°C. and 35°C., no change in yield of excess NPN was found. Incubation at 15°C to 18°C resulted in only about half as much excess NPN (Table IV). Further analysis showed that the amino nitrogen fraction of the

total NPN also increases after exposure to mustard. The increase is small but statistically significant. The increase of the amino N amounts to one half to two thirds of the corresponding figures for the total NPN. Since 30% of the amino acids which constitute the cell

TABLE IV

Total Non-Protein-Nitrogen after Varying Incubation Temperature

Exposure time to Mustard: 15 minutes.

Incubation time: 9 to 10 hours.

MICROGRAMS NITROGEN PER CORNEA					
Incubation Temperature	Before Incubation Control	After Incubation			
		A Control	B Exposed to Mustard	Difference B-A	Average Difference
15-18°C.	251.0	234.0	253.0	19.0	21.2
	281.0	238.6	267.4	28.8	
	233.0	230.7	248.7	18.0	
	251.7	259.3	280.8	21.5	
	275.0	262.0	280.6	18.6	
23°C.	271.0	251.0	280.5	28.5	28.5
28°C.	229.0	251.0	289.8	38.8	53.7
	183.0	203.0	272.8	69.8	
	272.0	257.0	301.0	44.0	
		245.5	313.0	67.5	
		260.5	318.0	57.5	
		236.3	290.5	54.2	
		263.0	317.0	44.0	
33-35°C.	243.1	244.9	291.0	46.1	46.7
	256.0	269.0	299.8	30.8	
	281.0	244.0	305.4	61.4	
	191.0	202.0	250.6	48.6	

proteins contain nitrogen in a form which is not determined by the van Slyke manometric method, and since the proteolysis does not necessarily proceed to completion, these figures would seem to indicate that the entire increase in NPN is due to proteolysis (Table V).

(2) *Fractionation of Epithelial Proteins.* In an attempt to find a clue for an explanation of this limited increase in the NPN level, it

seemed of interest to examine the corneal epithelium for changes in the state of the proteins. We measured the amount of protein extractable from normal and mustard treated corneas in various electrolyte solutions: 22% Na_2SO_4 , 10% NaCl , and 10% NaCl followed by $\text{N}/1$

TABLE V

Effect of Mustard on Total Non-Protein-Nitrogen and Amino-Nitrogen Content of Beef Corneas

Exposure to Mustard: 15 minutes.

Incubation: 10 hours at 28°C.

MICROGRAMS PER CORNEA			
Total NPN		Amino-N	
Controls	Exposed	Controls	Exposed
303	393	105	114
305	355	85	117
298	337	102	104
311	310	115	107
330	311	114	123
310	324	100	126
294	343	105	120
288	359	109	121
333	339	84	133
300	334	98	142
231	333	99	159
294	369	81	112
291	313	95	104
309	351	98	114
315	332	99	120
297	285	103	133
271	312	93	117
	307	81	133
		109	
Av.....299	334	99	121
Excess NPN.....	35	Excess Amino-N.....	22

NaHCO_3 . The time of exposure to mustard was 15 minutes followed by incubation at 29°C for 5-7 hours. During the extractions all materials were kept in the refrigerator at 5°C.

For the extraction with 22% solution of Na_2SO_4 six corneas were pooled in each sample and the extraction was extended over a period of

48 hours, replacing the first 50 ml. portion of the extraction fluid after 24 hours. To the collected extract (total 100 ml.) we added 20 ml. of 50% trichloroacetic acid and allowed it to stand for 12 hours. The precipitate was collected by centrifugation, the supernatant was discarded and the precipitate dissolved in concentrated H_2SO_4 . An aliquot was used for determination of nitrogen.

In several experiments samples containing three corneas were first extracted with 10% NaCl solution for two 24 hour periods using 10 ml. and 5 ml. portions which were collected by decantation. At the end of the second day the epithelium became so loose that it could easily be removed in sheets with a forceps. The separated epithelium was transferred to 12 ml. centrifuge tubes and the extraction of the separated epithelium was continued, daily adding a fresh 2 ml. portion of the 10% NaCl solution for five more times, and separating the extracts by centrifugation. The collected extracts were precipitated with 20% trichloroacetic acid. In one series the extracts were saturated with MgSO_4 . After 12 hours a small precipitate developed which was filtered off. The collected precipitate was dissolved by washing the filter with several portions of physiological saline until no further protein came into solution. Aliquots of the pooled washings were used for nitrogen determinations. In another series in which the corneas had been extracted with 10% NaCl we continued the extraction of the residues for three days with 2 ml. portions of a N/1 solution of NaHCO_3 . These extracts were also precipitated with trichloroacetic acid and the precipitate dissolved in concentrated H_2SO_4 for N determination.

Results. The extraction with Na_2SO_4 and with NaHCO_3 yielded very small amounts of protein: A considerable yield was obtained on extraction with 10% NaCl solution. We noticed, however, that the yield of proteins obtained in the first two days of the extraction of the whole intact cornea was very small. The bulk of the precipitable material was extracted from the isolated epithelium in the first two days following the separation. The assumption that most of the protein in our extracts is derived from epithelial cells is corroborated by the fact that the sum of the proteins in the extracts plus the protein in the extracted epithelial residue agrees closely with the amount of total protein calculated from the average freshweight of the epithelium

(about 12 mg. protein per cornea). Comparing the figures for the amount of protein extracted with 10% NaCl, and for the protein in the epithelial residue, we find that more than half of the proteins of the epithelial cells are extracted.

No significant differences could be observed between the fractions which were obtained from normal corneas and from corneas after exposure to mustard (Table VI). However, it should be pointed out that

TABLE VI

Protein Content of Extracts of Normal Corneas and of Corneas after Exposure to Mustard

Exposure to Mustard: 15 minutes.

Incubation: 5-7 hours at 29°C.

The figures give mg. protein (mg. nitrogen \times 6.25) extracted per cornea.

EXTRACTION WITH 22% Na ₂ SO ₄ PRECIPITATION WITH TRICHLORACETIC ACID										AVERAGES
Controls	2.0	2.1								2.05
Exposed	1.9	2.1								2.0
EXTRACTION WITH 10% NaCl, PRECIPITATION WITH TRICHLORACETIC ACID										
Controls	8.0	7.8	8.0	7.2	9.5	8.6	8.8	7.1	7.8	8.1
Exposed	8.0	8.0	7.1	8.0	8.3	9.6	8.0	7.7	7.4	8.0
EXTRACTION WITH 10% NaCl, PRECIPITATION BY SATURATION WITH MgSO ₄										
Controls	0.8	0.8								0.8
Exposed	0.7	0.8								0.85
EXTRACTION WITH NaHCO ₃ AFTER PRELIMINARY EXTRACTION WITH 10% NaCl PRECIPITATION WITH TRICHLORACETIC ACID										
Controls	0.8	0.6								0.7
Exposed	0.7	0.7								0.7
Residue after extraction with 10% NaCl.....										6.0

previous calculations indicated that the observed increase in non-protein nitrogen would correspond to the proteolysis of not more than 1% of the total protein in the epithelium. It would be necessary to devise finer methods of fractionation or to carry out larger series of experiments to detect differences of this order of magnitude. An experiment in which we attempted to measure the adsorption of trypsin on the surface of corneas likewise failed to show any difference between mustard exposed tissues and controls, perhaps also because of inadequate sensitivity of the method.

(3) *Utilization of Ammonia.* Injected ammonia (supplied as ammonium succinate) is utilized at a rate of about 5 micrograms per hour per cornea (Table VII). Simultaneous determinations of the total non-protein nitrogen (NPN) revealed a loss equivalent to the disappearance of the ammonia, indicating that ammonia or an equivalent amount of some other NPN factor is synthesized into a non-extractable constituent of the cornea. This observation gives increased significance to the results which we obtained on examination of the effect of

TABLE VII

Utilization of Ammonia Nitrogen in Beef Corneas on Incubation

Incubation: 10 hours at 28°C.

Ammonium succinate injected: about 200 micrograms N per cornea.

MICROGRAMS PER CORNEA							
Ammonia Nitrogen				Total NPN			
Control		Ammonium succinate injected		Control		Ammonium succinate injected	
Before	After	Before	After	Before	After	Before	After
Incubation		Incubation		Incubation		Incubation	
20.8	24.5	143.5	100.8	277.5	258	418	315
21.5	27.3	141.5	86	287.5	231.5	405.5	296
28	35.8	145.5	83.1	252	232.3	384.8	266.3
33	33.7	158.6	105.4	301.6	272	486	401
19.8	20.8			303.6	222.6		
				270	226.3		
				264.8	261.8		
Av... 24.6	28.4	147.3	93.8	279.6	243.5	423.6	319.6
Change +3.8		-53.5		-36.1		-104	

mustard on the ammonia utilization. In these experiments we found no inhibition of the disappearance of ammonia after an exposure of the corneas for 15 minutes to mustard vapor (Table VIII). These negative results demonstrate that some synthetic processes in the tissue are not interfered with by a dose of mustard which causes severe clinical symptoms in the cornea in vivo, and is accompanied by a definite damage to the epithelial cells in the excised tissue and inhibits certain metabolic reactions such as lactate disappearance.

Comparing the averaged figures given here with those in Table I

some divergence will be noticed. In these experiments a slight decrease of the NPN in the incubated control corneas was observed while in the previous series of experiments no significant change could be detected. It is possible that these are real differences which occur in different batches of corneas. It should be pointed out, however, that the present series of experiments include a smaller number of samples and that a deviation of the average value could be due to the wide scattering of the individual samples. Also, it should be pointed out that the control corneas in this series of experiments were injected with saline solution, whereas the corneas in the previous experiments were

TABLE VIII

Effect of Mustard on the Utilization of Ammonia Nitrogen
Ammonium succinate injected; about 200 micrograms N per cornea.
Incubation: 10 hours at 28°C.
Exposure time to Mustard: 15 minutes.

Before Incubation	AMMONIA NITROGEN IN MICROGRAMS PER CORNEA	
	After Incubation	
	Controls	Exposed to Mustard
151.5	86.2	90.4
154.4	89.9	95
148.8	88.1	94.7
151.0		
151.4		
Av.....151.4	88.1	93.3

incubated without injected solution, and that this might have an influence on the formation and disappearance of non-protein nitrogen. At any rate our present conclusions are not affected by this apparent discrepancy.

(4) *Utilization of Serine.* Serine is not only an important constituent of most proteins; it has been found to function as precursor in the synthesis of phospholipoids, and its chemical constituents suggests a possible relation to carbohydrates, especially amino-sugars. The selection of serine as the first (and so far, the only) amino acid to be tested was prompted in addition by the availability of a specific and sensitive method for serine determination.

Results. Preliminary tests on trichloroacetic acid extracts of corneas showed that a substance is present in the cornea which gives the Boyd Logan color reaction and which if calculated as serine amounted to 90 micrograms per cornea. The nature of the substance was not investigated more closely and for convenience it will be designated as F.P. (formaldehyde producing). Determinations in extracts from separated epithelium and stroma demonstrated that the epithelium contains twice as much of this material as does the stroma. The wet weight of the epithelium is not more than 15-20% of that of the whole

TABLE IX

Determination of Endogenous "Serine" (F.P.) in Beef Corneas

Incubated 10 hours at 37°C.

Exposed to Mustard vapor for 15 minutes.

Calculated as Serine in micrograms per cornea.

CONTROLS		EXPOSED TO MUSTARD	
Stroma	Epithelium	Stroma	Epithelium
35.1	69.1	74.6	48.5
23.2	51.9	42.6	32.5
26.2	44.0	44.0	30.3
29.3	64.7	64.0	50.0
33.2	71.7	47.2	47.2
Av.....29.6	60.3	56.5	41.7
Whole cornea89.9		98.2	

cornea. The concentration of F.P. is, therefore, about 10 times higher in the epithelium than in the stroma.

On incubation of the corneas the total amount of F.P. remains practically unaltered both in normal samples, and after exposure to mustard. In the exposed samples, however, a marked change can be observed in the distribution of F.P. between the tissue components. In the period of incubation which we used, the ratio of concentration of F.P. in epithelium to that in stroma dropped from the normal level of 10:1 to less than 4:1 in corneas which had been exposed to mustard but remained unchanged in incubated control samples (Table IX).

Since the concentration of F.P. in the cornea is relatively low and

remains constant during incubation we concluded that the utilization of serine might be investigated if a relatively large amount of this substance was injected into the tissue. The amount of injected serine used in the following experiments was 10-20 times the amount of endogenous F.P. On incubation of such injected tissues at 37°C. serine was found to disappear in normal corneas at a rate of about 25 micrograms per cornea per hour. After exposure to mustard this rate drops to 7 micrograms per cornea per hour (Table X).

TABLE X

Effect of Mustard upon the Utilization of Serine by Beef Corneas

Amount injected: 2 mg. serine per cornea.

Determinations made on pooled samples of 3 corneas each.

Results in micrograms per cornea.

BEFORE INCUBATION		INCUBATED FOR 10 HRS. AT 37°C.			
Controls	Injected	Unexposed		Exposed to Mustard	
		Controls	Injected	Controls	Injected
88	897	79	673	93	857
93	895	78	690	91	833
	1120	77	957	120	1100
	1097	92	880	93	1107
	1120	84	793		957
	1119		800		980
Av. per Cornea.....	91	82	799	99	972
Serine Utilized			242		69

The rate of disappearance of serine was also followed by manometric measurements of amino nitrogen in the extracts. These two sets of measurements yielded the same rate of disappearance of injected serine, and indicated that in the utilization of serine its amino nitrogen is transferred to some non-extractable component of the tissue, while any non-nitrogenous residue is so altered as to be no longer oxidizable to formaldehyde by periodic acid (Table V).

Serine is the first nitrogenous compound, the utilization of which we have found impaired after exposure to mustard. Due to the slow rate of normal utilization of serine by the cornea, we have so far been unable to isolate any of its breakdown products, and no data are available which would suggest the mechanism of its utilization. It is plain,

however, that the serine nitrogen is transferred to some nonextractable tissue component, and that the process involves more than the simple oxidative de-amination of serine, for hydroxypyruvic acid, the product of such a reaction, would give formaldehyde on oxidation with periodic acid and hence be titrated as serine.

We are unable to throw any light on the endogenous substance which yields formaldehyde on oxidation with periodic acid. Exogenous serine is utilized in the cornea at an appreciable rate whereas the endogenous F.P. does not disappear from the tissue on incubation. It may well be that this endogenous material is normally produced as rapidly

TABLE XI

Utilization of Serine as Determined by Amino-Nitrogen Measurements

Amount injected: 2 mg. serine per cornea.

Results in micrograms nitrogen per cornea.

BEFORE INCUBATION		AFTER INCUBATION	
Controls	Injected	Controls	Injected
113.5	322	118	271
109	306	94.8	282
107	312	92.5	272
110	300	84	274
	308	108	258
			269
Average 109.9	309.6	99	271
Injected minus Controls.....	199.7		172.1

Utilized per hour: 2.76 micrograms nitrogen or 21.0 micrograms serine.

as it is consumed and that its level is thus kept constant on incubation. Since the utilization of serine is impaired after exposure to mustard, one might expect endogenous serine to accumulate in the tissue after exposure, but this is not necessarily so because the mustard injury may inhibit the formation of serine as well as its consumption. In any case, the inhibition of utilization of serine does not account for the increased NPN found after exposure to mustard.

SUMMARY AND DISCUSSION

The stability of the extractable non-protein nitrogen content of beef corneas on prolonged incubation was a gratifying finding, since it indi-

cated that the tissue was not undergoing autolysis. Indeed, the capacity of the tissue to utilize ammonia shows that synthetic processes are still going on in the cells. These findings strongly supported our previous conclusions that, with the simple techniques used, the corneas could be maintained alive for many hours in the incubator.

The extractable non-protein nitrogen content of the cornea increases after exposure to mustard. Approximately one half to two thirds of the increased NPN is titratable as amino nitrogen, indicating that the excess NPN is derived from proteins. We have attempted to demonstrate the presence of denatured proteins in the tissue after exposure to mustard, but the available methods proved insufficiently sensitive to give us an answer to this question.

Certain quantitative relations between the amount of mustard taken up by the cornea and the amount of NPN released are of interest. With exposures of 10 minutes we estimate that 6 micrograms of mustard is taken up per square centimeter of cornea, or 24 micrograms per cornea. Approximately one sixth of this, or 4 micrograms, is bound by the tissue. This exposure results in the formation of about 20 micrograms of NPN or, roughly, 70 moles of nitrogen for each mole of mustard bound. This is roughly the order of magnitude of accessible SH groups to nitrogen in some proteins.

The failure of the excess NPN to increase indefinitely with increased dosage of mustard raises interesting problems. It may be supposed that with increased exposure some proteolytic enzymes are inactivated. However, this should result in greater final NPN production with greater exposure to mustard even if the rate of production of the excess NPN were slowed. This we did not find. In a short series of experiments we compared the NPN production after exposure to mustard on aerobic and anaerobic incubation. Under anaerobiosis proteolytic enzymes are generally activated, but the excess NPN produced under these conditions was somewhat less than on aerobic incubation. It would seem possible, therefore, that oxidation of some proteins that had reacted with mustard may enhance their susceptibility to hydrolysis. This suggestion is made in order to show a possible relation between the excess NPN formed on incubation after exposure to mustard and the loosening of the corneal epithelium which occurs after exposure. The dosage range eliciting these two effects is identical. The increased

NPN appears before the loosening of the epithelium. The cohesive boundary can be destroyed by proteolytic enzymes. Anaerobic incubation of mustard exposed corneas protects the tissue against loss of cohesion.

The effect of mustard on serine metabolism revealed many points of similarity to its effect on lactate metabolism. Both metabolites are normally consumed by the intact cornea but not by the denuded stroma, and the consumption of each is inhibited after exposure to mustard. The endogenous formaldehyde producing material which we have titrated as serine is normally present in far higher concentrations in the epithelium than in the stroma. After exposure to mustard the concentration of this material rises in the stroma and falls in the epithelium. A possible explanation of this finding would be in inhibition of the transfer of this substance from stroma to epithelium. We have performed one preliminary experiment to find out whether there is a corresponding active transfer of exogenous serine. After injection of serine into the stroma, and subsequent incubation, the concentration in the epithelium was found to rise rapidly reaching a level far higher than that in the stroma. The calculated concentration of serine in the epithelium in this experiment remained much higher than that in the stroma even after subtracting the normal titration values of the endogenous formaldehyde producing substance.

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XVI. COMPARISON OF THE EFFECTS OF MUSTARD, ULTRAVIOLET AND X-RADIATION, AND COLCHICINE ON THE CORNEA*

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The analysis of the effects of mustard and the nitrogen mustards reported in the preceding papers cannot fail to raise in the mind of the reader many questions as to the general similarity between the tissue reactions to these agents and those following X-ray and ultraviolet irradiation. To a lesser extent similarities may be noted to the reactions following the administration of colchicine. Equally striking similarities exist between the effects of these agents in producing erythema, vesication and ulceration of the skin. The nature and distribution of the visceral lesions following systemic administration of the mustards is remarkably similar to that produced by exposure to X-ray and other penetrating radiations.

In spite of these similarities some differences in the tissue reactions to these various agents are obvious, and a detailed quantitative analysis on a single tissue would seem desirable. For this purpose the cornea possesses many advantages, both in accessibility to the various agents, and in respect to the availability of quantitative methods for the study of various effects. The data available for such a comparison are, unfortunately, by no means complete. They suffice, however, to indicate some specific differences between the effects of several of the agents.

The cytological studies in regard to mitotic and nuclear changes were performed mainly on rats but have been amplified for the present purposes by studies on beef corneas. There is a general similarity in the reaction of the two species, but some minor differences will be noted below. Quantitative studies on the loosening of the corneal epithelium were performed on beef corneas. Less exact methods applied to the study of this phenomenon on rats' corneas revealed no differences. The analysis of metabolic effects was made exclusively on

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beef corneas. In addition to the data on the metabolic effects of mustard on the cornea presented in the preceding papers, a study of the non-protein nitrogen content of the tissue after exposure to ultraviolet light and to colchicine is reported below.

AGENTS TO BE COMPARED

1) *Sulfur and Nitrogen Mustards.* Studies on the altered metabolism of the cornea after exposure, reported in the preceding papers, have been limited entirely to sulfur mustard, and no data are available concerning the effects of the nitrogen mustards on corneal metabolism. Studies on mitotic and nuclear changes and on loosening of the epithelium revealed no differences between mustard and three of the nitrogen mustards.

Estimates of the amount of mustard absorbed by the tissue in the production of a given effect must be given with considerable reserve. In respect to those doses which produce the metabolic disturbances and the epithelial loosening in the beef cornea, we are on reasonably solid ground, having measured directly the mustard uptake of the beef cornea under the conditions of exposure that we were using. In order to reach an estimate of the dose required for mitosis inhibition two extrapolations were necessary. In the first place, we have assumed that the uptake of mustard per square centimeter in the rat's cornea under similar conditions of exposure is the same as in the beef, and have then measured the time of exposure to mustard vapor required to produce a lesion of given clinical severity in the rat. We have then reproduced lesions of the same clinical severity by administering to the eye a drop of mustard dissolved in hexane. Using instillation of much more dilute solutions of mustard in hexane we have explored the effect on inhibition of mitosis. The estimate we have given for the dosage related to different degrees of mitosis inhibition rests on the assumption that the amount of mustard absorbed from a hexane solution, or from vapor, are the same if they cause lesions of the same severity; and that the amount absorbed from increasingly dilute solutions of hexane is proportional to the concentration. Comparison of rat and beef corneas exposed to the same doses of mustard vapor indicates that the rat's cornea is about twice as susceptible to this agent in respect to loosening of the epithelium as the beef.

2) *Ultraviolet Light.* A report of many of our findings on the effect

of ultraviolet light on the rat's cornea has been published elsewhere (1). Some additional data relating to the effects of specific wave lengths have been obtained in collaboration with Dr. Kinsey at the Howe Laboratory and later with Dr. Hollaender at the National Institute of Health and will be included in brief summary below.

A comparison of the effects of ultraviolet light on beef and rat corneas in producing epithelial loosening and nuclear fragmentation revealed that in order to elicit comparable reactions, a dose was required for beef tissue 10–20 times that required for the rat. Moreover, nuclear fragmentation develops much more slowly in the beef than in the rat after both ultraviolet and mustard exposures. In the rat's corneal epithelium, clumping of the nuclear chromatin is visible 2–3 hours after exposure, and disappearance of the nuclear membrane with dispersal of the chromatin into the cytoplasm is abundantly shown after 6 hours of incubation. In the beef, clumping of the nuclear chromatin is visible after 6 hours at 37°C., but in most of the affected cells the nuclear membrane remains intact up to 18 or 20 hours after exposure. The chromatin in the affected cells in the beef usually forms a solid mass in the center of the nucleus surrounded by an eosinophilic zone, or, alternatively, it is adherent to the nuclear membrane. Similar patterns are seen occasionally in the rat's corneal epithelial cells. Unless otherwise specified, the dosage of ultraviolet was controlled by varying the time and distance of exposure, using a cold quartz mercury arc as the source. At the standard distance of 5 cms. this source yielded approximately 10^6 ergs/cm² per minute.

3) *X-ray, Grenz ray, Beta ray.* A comparison of the effect of hard (200 KV) and soft (24 KV) X-ray was made in collaboration with Dr. Gustav Bucky. For equal exposure in terms of roentgen units (ionization produced per cm²) equal effects were produced. Beta ray from radon contained in a thin glass capsule was applied with the capsule 5 mm. from the apex of the cornea. The results were essentially similar to those with X-rays. Under the conditions of our experiments, exposure to Beta radiation equal to 1 gram second (radium) gave an inhibition of mitosis equal to that of approximately 50 roentgen units. Owing to the geometrical relations between the applicator and the cornea, the exposure to Beta rays at the periphery of the cornea was slightly less intense than at the center, and recovery from the inhibition

began sooner at the periphery. Full report of our experiments with these ionizing radiations will be given elsewhere.

4) *Colchicine*. Results on mitotic activity in the corneas of rats following systemic administration of colchicine have already been reported (2). The results of intracorneal injections are given below.

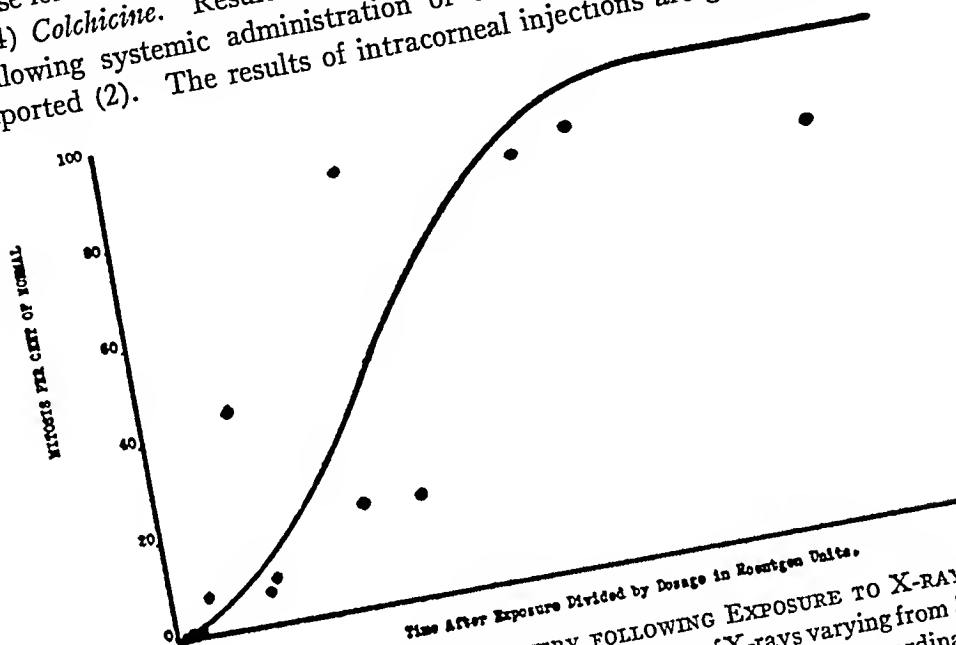


FIG. 1. MITOSIS INHIBITION AND RECOVERY FOLLOWING EXPOSURE TO X-RAYS
The curve is a composite of results obtained with doses of X-rays varying from 50 to 2000 r units. The curve is plotted with mitosis percent of normal as ordinate versus time after exposure divided by dosage in r units as abscissa. Each dot represents the average of counts of 2-4 animals.

EFFECTS

I. *Mitosis Inhibition*. With X-ray over a range of exposures from 50 to 2000 roentgen units, inhibition of mitosis was immediate and complete. Similar results were obtained with Beta rays. Recovery of mitotic activity followed an S shaped (autocatalytic) curve as shown in Fig. 1. With increasing doses, the slope of the recovery curves was flatter, and the time to 50% recovery was proportional to the dose. Since mitotic activity was initially completely inhibited with doses that produced varying inhibitions of the recovery mechanism, it seems possible that X-rays injure two components of the mitotic mechanism: (A) a component immediately necessary for mitosis and (B) a

component required to recover from the inhibition. (B) may, for instance, be required to form (A).

With ultraviolet and mustards, the inhibition of mitosis developed slowly over the course of some hours, the speed with which the inhibition developed being roughly proportional to the dose (Fig. 2). Recovery from the inhibition followed curves similar to those obtained

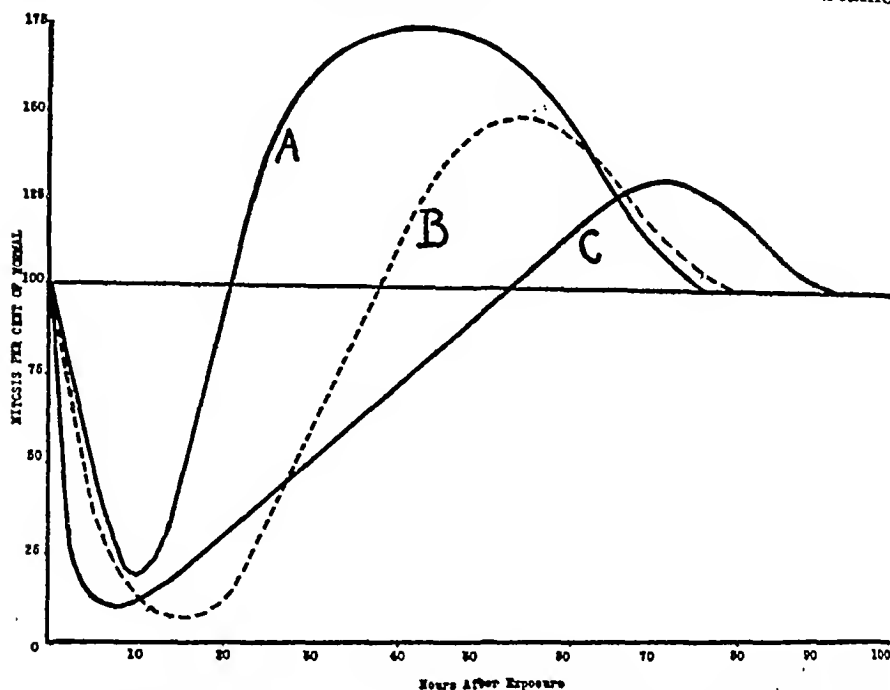


FIG. 2. MITOSIS INHIBITION AND RECOVERY FOLLOWING EXPOSURE TO TWO DIFFERENT DOSES OF ULTRAVIOLET (A AND C) AND TO A SINGLE DOSE OF NITROGEN MUSTARD (B)

The dose of Ultraviolet producing the results shown in Curve C is double that of Curve A. Curve B is the same as that shown in a previous report (4).

with X-rays. Comparing inhibitions of equal duration produced by ultraviolet and mustard on the one hand with those produced by X-rays on the other, it was found that with mild exposure (6-12 hours inhibition) the inhibition was less complete with ultraviolet and mustard than with X-ray.

If the supposition about a possible dual injury with X-ray is correct, then ultraviolet and mustard do not injure the (A) component but may injure the (B) component similarly to X-rays. The matter, however,

is more complicated. Over the range of dosage we have studied, the inhibitions produced by ultraviolet and mustard are quite regularly followed by a period of mitotic activity exceeding that of the control, while we have observed no such recovery overshooting following X-radiation. On the other hand, recovery overshooting has been observed following mitosis inhibition from X-radiation on grasshopper neuroblast (3). Perhaps we have not yet reached sufficiently low doses of X-radiation to demonstrate recovery overshooting in the cornea, but in any case inhibitions of similar durations are associated with recovery overshooting in the case of mustard and ultraviolet, and are without this in X-radiation.

Several alternatives present themselves as possible explanations of the recovery overshooting. Some normally inhibitory mechanism may be removed, or some necessary metabolite may accumulate in excess. This matter has already been discussed in a previous paper (4) where evidence was presented indicating that during the overshooting following mustard there was no disproportionate excess of cells in the premitotic state. Similar results have been obtained on the overshooting following ultraviolet. The recovery overshooting following mustard and that following ultraviolet radiation was tested with adrenalin, and it was found that the mitotic activity was just as susceptible to inhibition by adrenalin during the overshooting as in normal controls.

II. *Nuclear Fragmentation.* The phenomena of nuclear fragmentation following exposure to mustard (5) and to ultraviolet (1) have been reported in previous papers. In respect to the cytological pattern of development, no differences were noted between the two agents when studies were made on the same species. The incubation periods and the time of development through various stages were the same. In both cases the development of nuclear fragmentation was inhibited by anoxia and by reduced temperature of incubation. In each case evidence has been adduced suggesting that the nuclear fragmentation represents, perhaps, a pathological and distorted form of mitosis.

In spite of the considerable similarities between nuclear fragmentation produced by mustard and by ultraviolet, important differences are still present. Under normal conditions only a few cells, and these only in the basal layers of the corneal epithelium, are susceptible to nuclear

fragmentation by mustard and nitrogen mustard. We have suggested that these susceptible cells may be those which are in a premitotic state (5). A variety of conditions, e.g. previous exposure to adrenalin, to hypotonic, and to acid solutions, greatly increase the number of susceptible cells, and under these conditions cells in the superficial, normally non-mitosing layers may also show nuclear fragmentation after exposure to the mustards.

In contrast to these findings ultraviolet produces fragmentation mainly in the superficial layer of corneal cells, and, in the rat, cells showing this reaction are regularly abundant, irrespective of the conditions of pretreatment so far tested. It should be noted that mitotic activity is normally absent in this cell layer. It might be supposed that exposure to ultraviolet entails an effect sensitizing cells to nuclear fragmentation similar to that of the various agents which were shown to be sensitizing when followed by mustard. We are forced to the conclusion that while the pathological processes of nuclear fragmentation produced by ultraviolet and by mustard are apparently identical, the state of cells responding in this way to the injury is different for the two agents, and hence that the primary injuries by these two agents are different.

In the corneal epithelial cells we have seen no similar nuclear fragmentation following X-ray. We have not, so far, tested whether the pretreatments which sensitize the corneal cells to nuclear fragmentation by mustard have a similar effect in relation to X-ray. It may be noted that nuclear fragmentation in the corneal epithelium is associated with a marked increase in the amount of Feulgen positive material similar to that seen in normal mitosis. Hevesy (6) has recently reported that in certain tissues which he has studied, mitosis inhibition produced by X-ray is associated with an inhibition in the synthesis of desoxyribose-nucleic acid. An inhibition of desoxyribose-nucleic acid synthesis might make nuclear fragmentation impossible since in the cornea this phenomenon is associated with the accumulation of Feulgen positive material. It may be noted, however, that nuclear fragmentation occurs in the bone marrow and in lymphoid tissue after X-radiation. No information is available as to whether, in the tissues, nuclear fragmentation is also associated with the synthesis of Feulgen positive material.

It may be pointed out that after exposure to X-ray and Beta ray pathological mitoses in the form of unequal separation of the chromosomes at anaphase, or multipolar mitotic spindles are occasionally seen. Phenomena of this type have been frequently described in relation to the effects of ionizing radiations on other tissues. They appear to us to have no relation to what we have defined as nuclear fragmentation, and we have not seen mitoses of this type following mustard or ultraviolet exposures.

Further evidence that mitosis inhibition and nuclear fragmentation result from injuries that are at least to some extent independent is seen in Table I. Taking the dose which produces 24 hours inhibition of mitosis as the basis of comparisons, nuclear fragmentation is produced by one third this dose in the case of ultraviolet, ten times this dose in the case of mustard, and not with two hundred times this dose in the case of X-ray.

Colchicine does not inhibit the onset of mitosis in the corneal epithelium in the doses that we have studied, but stops the process in metaphase without spindle formation. After prolonged arrest in metaphase the chromatin tends to clump irregularly and eventually fragments become dispersed in the cell. The final result is similar to that produced by mustard and ultraviolet. Since only spontaneously mitosing cells reach this state under colchicine the lesion is confined to the basal epithelium. The time required for a cell in arrested metaphase to undergo this form of chromatin dispersal is markedly influenced by the temperature. If rats' eyes are removed 40 minutes after systemic colchicine injection (50 mg. per Kg. bodyweight) and incubated in moist chambers, the chromatin dispersal occurs in 4 hours at 38°C., in 8 hours at 30°C., and it only begins to appear between 20 and 24 hours at 25°C.¹

III. *Loosening of the Corneal Epithelium.* Ionizing radiations, ultraviolet, mustard, and colchicine, all produce loosening of the corneal epithelium, but at doses that vary widely relative to the mitosis inhibiting dose. The two effects appear, therefore, to be independent. The question of the possible relation between nuclear fragmentation

¹ It is of some interest in this connection that the systemic toxic effects of colchicine have been reported to be markedly dependent on temperature in hibernating (7), and in cold blooded animals (8).

and epithelial loosening in the case of mustard has already been discussed (4). Both processes are suppressed by anoxia and hence both require oxygen for their development, but the temperature coefficient of nuclear fragmentation is steeper ($Q_{10} = 3.5$) than that for loosening of the epithelium ($Q_{10} = 3$), consequently either phenomenon can be made to precede the other depending on the temperature of incubation. Moreover, under basal conditions (absence of preceding adrenalinemia) *in vivo*, nuclear fragmentation is minimal but the loosening process is not diminished.

We believe, therefore, that the epithelial loosening process is independent of nuclear fragmentation in spite of the fact that there are some apparent correlations between the two processes. For instance,

TABLE I
Comparison of Dosage Level with Different Agents in Rats' Corneas

	MUSTARD MICROGRAM/CM ²	ULTRAVIOLET EXPOSURE TIME, MINUTES	X-RAY ROENTGEN UNITS
24 hours Mitosis Inhibition.....	0.01	1½	100
Threshold Nuclear Fragmentation.....	0.1	½	
Loosening of the Epithelium.....	1.0	3	20,000*

* The loosening of the corneal epithelium occurs as part of the late ulcerative reaction and must be only very indirectly related to the initial ionizing effect of the radiation.

ultraviolet, which causes the most abundant nuclear fragmentation, produces epithelial loosening at relatively low doses, while X-ray which fails to cause nuclear fragmentation, requires the highest dose to produce epithelial loosening when the dose for mitosis inhibition is taken as the standard of reference (Table I).

Colchicine administered systemically in doses fatal to many of the rats in 18-24 hours (2 mg./kilo) fails to produce loosening of the corneal epithelium. Intracorneal injection of as little as 0.1 micrograms in beef corneas produced definite loosening of the epithelium (Table II). Since the wet weight of the beef cornea is about one gram this amounts to a concentration of 0.1 milligrams per kilo. It follows that either the beef cornea is much more susceptible to the loosening effect of colchicine than the rat's, or that systemically injected colchicine in the rat

does not achieve a uniform distribution throughout the animal.² Since some colchicine arrested mitoses appear in the beef cornea with injections of 1 microgram of colchicine into the isolated tissue, the loosening of the epithelium by this agent has no direct relation to inhibition of mitosis.

IV. *Increased Non-Protein Nitrogen.* In the preceding paper (9) experiments were reported showing that after exposure to mustard there is an increase in extractable NPN in the corneas. Using the

TABLE II
Effect of Colchicine upon the Adhesion of the Corneal Epithelium

Dose: injected in 0.25 ml. of 0.3% saline.

Incubation: 10 hours at 37°C.—A; 10 hours at 28°C.—B.

AMOUNT INJECTED	A*	B*
Controls	-----±-	-±
0.1 micrograms	-++	+±
1.0 micrograms	+++	+±
10.0 micrograms	+++++	++
100.0 micrograms	+++++	+±
500.0 micrograms	+++	+±
1.0 micrograms	+++	±±
	+++	±±

* Each symbol in these columns represents an experiment on a beef cornea. The epithelial adhesion was tested with a scraping weight of 90 gms.
- indicates less than $\frac{1}{3}$ of epithelium removed.
± indicates about $\frac{1}{3}$ of epithelium removed.
+ indicates more than $\frac{1}{3}$ of epithelium removed.

same techniques, a series of experiments were performed comparing the effect of mustard with that of ultraviolet light and of colchicine. After exposures of ultraviolet light capable of producing loosening of the epithelium, an increased NPN content was found similar to that following similar exposures to mustard (Table III). The time of development, the amount of increase, the proportion of amino nitrogen in the NPN, and the temperature dependence of the effect was similar with these two agents. These results support our previous conclusion

² The colchicine preparation which we used was the usual commercial one. The epithelial loosening which we observed following injection of this material may be due to some impurity present and not to the colchicine itself.

that the increased NPN is not derived from cells undergoing nuclear fragmentation for such cells are much more abundant after ultraviolet than usually the case after mustard.

No change in the extractable NPN content of the cornea was noted after injections of colchicine (Table IV). If the increased NPN after

TABLE III

Effect of Ultraviolet Irradiation upon the Non-Protein-Nitrogen Content of Beef Corneas

Treatment: irradiated for 30 min. at a distance of 2cm. from source.

Incubation: 6 hours at 37.5°C.

MICROGRAMS NPN PER CORNEA	
Controls	Treated
247	273
239	283
252	297.5
253.5	274
259.5	321
267	336.5
257.5	302.5
251.5	282
275.5	296.5
219.5	313
216.5	258.5
245	299.5
244.5	229.5
232.5	276.5
269.5	246
291	275
261.5	267.5
277.5	263
Average.....249	277

Average increase per cornea = 28 micrograms NPN.

mustard is connected with the loosening of the corneal epithelium, then the mechanism of loosening of the epithelium by colchicine must be different from that by mustard. Since nothing is known concerning the mechanism of action of colchicine in chemical or metabolic terms, no interpretation of this negative finding can be offered at the present. A comparison of the effects of mustard, ultraviolet, and colchicine on the beef corneas is shown in Table V.

TABLE IV

Effect of Colchicine upon the Non-Protein-Nitrogen Content of Beef Corneas

Amount injected: 10 micrograms colchicine in 0.25 ml. of 0.3% saline.

Incubation: 10 hours at 37°C.

MICROGRAMS NPN PER CORNEA	
Controls	Treated
278	290
295.5	283.5
201	286
229.5	247
238	232
248	224.5
238.5	243
246	241.5
276.5	228.5
271.5	242
263.5	247
	276.5
Average.....253	253.5

TABLE V

Relation between Proteolysis, Epithelial Loosening, and Nuclear Fragmentation in Beef Corneas

TREATMENT	CONDITIONS OF INCUBATION	PROTEOLYSIS (increase in NPN)		LOOSENING OF EPITHELIUM*		NUCLEAR FRAG- MENTATION IN EPITHELIUM†	
		Aero- bic	Anaer- obic	Aero- bic	Anaer- obic	Aero- bic	Anaer- obic
1. Exposed to Mustard Vapor: 15 min. Uptake 0.6 micrograms per cm. ² per minute	10 hours at 28°	+	+	+	—	—	—
	20 hours at 29°	+	+	+	—	±	—
	10 hours at 37°	+	+	+	—	±	—
2. Irradiated at 2 cm. distance from ultraviolet source: 30 min.	6 hours at 37°C.	+	—	—	—	±	—
	12 hours at 30°C.	—	—	—	—	±	—
	20–24 hours at 30°C.	+	—	+	—	—	—
3. Injected with 10 micrograms Colchicine	10 hours at 37°C.	—	—	+	—	±	—
	10 hours at 28°C.	—	—	±	—	—	—

* The adhesion of the epithelium was tested with a scraping weight of 90 gms. Significance of symbols same as in Table II.

† — indicates no nuclear fragmentation.

± indicates intranuclear chromatin clumping.

+

† Superficial layers.

SUMMARY

Some of the tissue reactions to X-ray, ultraviolet radiations, and colchicine, have been compared with similar reactions to mustard. Among the agents compared, the effect of ultraviolet resemble those of mustard most closely, though with definite quantitative differences and with differences in relative susceptibility among various layers of corneal epithelial cells. If the mitosis inhibition produced by X-ray has any element in common with that produced by ultraviolet and mustard, then the effects of both types of agents are complex, each involving some factors not shared by the other. In the case of ultraviolet and mustard there may be some connection between the factors which lead to mitosis inhibition and those which lead to nuclear fragmentation, but nuclear fragmentation was not found in the corneal cells after X-ray in spite of marked inhibition of mitosis. Neither mitosis inhibition nor nuclear fragmentation appears to be directly connected with the loosening of the corneal epithelium nor with the increased NPN content of the cornea found after ultraviolet or mustard exposure. If the increased NPN following mustard is connected with the mechanism producing loosening of the epithelium which occurs with the same intensities of exposure, then the mechanism of loosening the epithelium by colchicine is different from that induced by mustard.

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XVII. SUMMARY AND SOME POSSIBLE INTERPRETATIONS

JONAS S. FRIEDENWALD

A general summary and discussion of the effects of mustard on nuclear activities in the corneal tissues is given in the preceding paper. The present paper concerns the effects of mustard on corneal metabolism and the possible relation of these effects to the loosening of the corneal epithelium.

I. *Metabolic Interaction between the Corneal Epithelium and Stroma.* The evidence presented in the preceding papers reveals that the carbohydrate metabolism of the corneal epithelium is similar to that found in many other tissues. It has a cyanid sensitive oxygen uptake and contains cytochrome oxidase. It consumes glucose, glycogen, lactate, and pyruvate. Under aerobic conditions and adequate supply of carbohydrate, the oxygen uptake equals that required for complete combustion of the carbohydrate consumed. Under anaerobic conditions, lactate is produced equivalent to the loss of glucose and glycogen. Glycolysis is inhibited by fluoride and iodoacetate, and under these circumstances phosphate esters accumulate, indicating that the glycolytic system follows the usual pathways. In the absence of adequate carbohydrates, the oxygen uptake remains normal or rises. This is not associated with any increased non-protein nitrogen. Consequently, the alternative metabolites are probably fats.

In contrast with the conventional metabolic pattern of the epithelium, the stroma has no oxygen uptake and can utilize neither lactate nor pyruvate. Nevertheless, it is capable of utilizing glucose at a rate per cell about twice that of the epithelium. Lactate is produced by the stroma but cannot be consumed by it. In the isolated cornea the lactate produced by the stroma is utilized by the epithelium, and constitutes an appreciable fraction (perhaps about 25%) of the total carbohydrate supply of the epithelium. Moreover, the aerobic glucose consumption of the stroma with the epithelium in place appears to be considerably less than in the freshly denuded stroma, indicating a control of glucolysis in the stroma by the oxidative metabolism of the adjacent epithelium, analogous to the familiar intracellular Pasteur effect.

The denuded corneal stroma, however, rapidly loses its capacity to utilize glucose, and an examination of the distribution of phosphate esters in the tissue suggests that the failure in glucose consumption is due to an exhaustion of the capacity of the tissue to transfer phosphate from glycerophosphate to hexose. We have concluded, therefore, that the epithelium normally assists the stroma in maintaining a supply of carriers for energy-rich phosphate transfers.

After exposure of the cornea to mustard in appropriate dosage, the consumption of stroma lactate by the epithelium is markedly inhibited. This inhibition occurs even when the epithelium is still able to consume its endogenous stores of lactate at an almost normal rate. Consequently, the epithelium must possess a special mechanism for the utilization or transfer of the stroma lactate. In either case it is reasonable to suppose that this mechanism is located at, or close to the stroma-epithelium boundary. Failure in the utilization of stroma lactate after exposure to mustard is associated with a corresponding decrease in O_2 uptake, indicating that the metabolites (fats) which normally serve as alternates when the supply of lactate is reduced, cannot be consumed after mustard injury. If the same injury is responsible for inhibition of the utilization both of lactate and of its alternates, a link in the hydrogen transport system common to both of these metabolites must be damaged by mustard. This leads to the conclusion that a special hydrogen transport system is present for the utilization of the stroma lactate, independent of that concerned with the utilization of endogenous lactate in the epithelium.

II. Transfer of Metabolites from Stroma to Epithelium. Lactate, pyruvate, serine, and probably butyrate, are not consumed by the denuded stroma but are consumed by the cornea with the epithelium intact. The question, therefore, arises as to whether their penetration into the epithelium is by simple diffusion or by active transfer.

(1) *Pyruvate.* Cogan and his co-workers (1) have shown that the stroma-epithelium boundary is highly impermeable to ions, and his conclusions are strongly supported by our own measurement of the electrical resistance of the tissue (2). When sufficient pyruvate is placed in the stroma, the epithelium consumes 400 micrograms of pyruvate per hour. The movement of anions at this rate is equivalent to an electrical current of 0.1 milliamperes. The electrical resistance of the boundary is 500,000–1,000,000 ohms per square millimeter or

1,000 to 2,000 ohms per cornea. Consequently, energy equivalent to 0.1–0.2 volt would be required to transfer the pyruvate through the resistant boundary. The required energy seems excessive in relation to the diffusion potential of a 0.01 molar solution of pyruvate in the presence of physiologic concentrations of electrolytes.

As a matter of fact, Miles (3) has found a potential of approximately 0.001 volt associated with the corneal surface; but of the opposite sign to that required for the movement of anions from stroma to epithelium. Whether this potential arises in the cornea or in some of the intraocular tissues has not been decided. There is, furthermore, a difference in redox potential between epithelium and stroma, but if there is any redox interaction between epithelium and stroma in the cornea similar to that which has been found in some other tissues (4, 5), the compensating ionic exchange would be in the opposite direction to that required to transfer anions from stroma to epithelium.

In view of these difficulties we are compelled to consider the possibility that pyruvate may be converted into an uncharged molecular species in preparation for its transfer to the epithelium. One possibility is that the pyruvate might be esterified onto some component of the boundary layer, and de-esterified on the opposite side. A second possibility is that pyruvate which, as is well known, forms unusually stable condensation products, may be transferred to the epithelium in the form of an unionized polymer. Since the transfer and utilization of pyruvate remains unaffected after mustard, the precise mechanism of pyruvate transfer need not be pursued further.

(2) Lactate. The evidence against simple diffusion as the mechanism of transfer is considerably stronger in respect to lactate than pyruvate. The amount of stroma lactate consumed by the epithelium is only 25 micrograms per hour as compared with 400 micrograms pyruvate per hour, but the concentration of lactate in the epithelium except under very special experimental conditions is either equal to or higher than that in the stroma. In corneas freshly removed from the animals, the concentration of lactate in the epithelium is twice that in the stroma, and this difference can be maintained unchanged for many hours of incubation if glucose is supplied. If simple diffusion between the two tissues were possible equalization of the lactate content would be expected. Furthermore, the failure of

the stroma lactate level to rise significantly under these conditions indicates either that lactate is being transferred against an adverse concentration difference, or that the stroma has no glucose consumption. The latter alternative is contradicted by direct experimental measurements on the isolated stroma.

When excess lactate is injected into the stroma it is consumed at the rate of 25 micrograms per hour irrespective of its concentration in the stroma. This is, therefore, the upper limit of the activity of either the transfer or the consuming mechanism.

In spite of the strong evidence against transfer of lactate by diffusion, we have, so far, failed to obtain correspondingly direct positive evidence for active transfer. We have sought for conditions under which we could demonstrate a decline in stroma lactate during a period in which the concentration of lactate in the epithelium is higher than that in the stroma. Unfortunately, in fresh tissue at the time when the concentration difference is found, the cornea contains an appreciable reserve of glucose most of which is in the stroma. Until these reserves have been used up no decline in stroma lactate concentration is to be expected, since production of lactate in the stroma should balance its losses by transfer to the epithelium. By the time that the glucose reserves have been exhausted, and the stroma lactate level has begun to fall, the epithelium has also exhausted its quota of glucose and begun to consume its endogenous lactate. Consequently the concentration difference required to prove active transfer has disappeared. Moreover, in studying such a sequence of events, different corneas have to be sampled after different periods of incubation. Since individual variations in the lactate and glucose stores are considerable, rather large differences in the experimental results are required to be significant.

Indirect evidence of active transfer was, however, obtained through the demonstration that the transfer of stroma lactate, as well as its utilization, is inhibited by mustard. This is shown in experiments previously reported (6) in which, after exposure to mustard, the corneas were incubated anaerobically for several hours at room temperature. By this procedure the effects of the mustard injury are given time to develop while the lactate reserves in the tissue are maintained at a normal level. Subsequent aerobic incubation at 29°C enabled the

epithelium to consume its endogenous lactate and to produce a concentration ratio between the two tissues favorable to diffusion from stroma to epithelium. Nevertheless, the stroma concentration of lactate was fairly well maintained on many hours of incubation.

The evidence that mustard inhibits both the transfer and utilization of stroma lactate suggests that the two mechanisms involved may be closely linked. We have already suggested that the mechanism for utilizing the stroma lactate is at or near the stroma-epithelium boundary. If we assume that lactate is oxidized at the boundary and converted to pyruvate then the simultaneous inhibition of both the transfer and the utilization of stroma lactate by a single mustard injury would be explained. Similar oxidation of extracellular substrates on the stroma side of the stroma-epithelium boundary have been shown to take place in the ciliary body (4) and choroid plexus (5). In these organs the oxidative interaction across the boundary is associated with a secretory transfer of water and electrolytes. Such a transfer, however, depends on the nature of the boundary. No evidence of such a secretory transfer in the cornea is available, though Cogan's (7) finding that the corneal stroma is maintained in a state of detergescence when the epithelium is present, and swells by uptake of water in the absence of the epithelium, suggests that the interrelations of stroma and epithelium include some mechanism for the extraction of water from the stroma.

While the hypothesis suggested above regarding the mechanisms of utilization of stroma lactate appears to satisfy practically all the available data, there is one set of experiments not easily reconciled with this hypothesis. When iodoacetate is injected into the cornea, glycolysis is inhibited and the lactate reserves of the tissue are consumed in the presence of iodoacetate, even if a large excess of glucose is present. Mustard inhibits the consumption of the stroma lactate. If iodoacetate is injected into mustard poisoned tissues, the inhibition of lactate consumption is partially reversed. This apparent reactivation of an inhibited system by a second poison constitutes a contradiction of some of the assumptions we have made.

A way out of this dilemma is the following: we have assumed so far that glucose is the only possible source of stroma lactate, and that when the reserves of glucose are exhausted no further lactate production takes place in the stroma. Consequently, we have assumed

when the lactate level in the stroma remained stationary after exposure to mustard that the utilization of stroma lactate was completely inhibited. It may well be, however, that the stroma is able to produce lactate from endogenous stores other than glucose,—for instance glycogen or mucoid,—and that the inhibition observed after exposure to mustard would then represent a balance between the partially inhibited lactate consumption mechanism and the reduced lactate production in the absence of glucose. If this residual lactate production is now inhibited by iodoacetate, a resumption of the decline in stroma lactate would occur, producing an apparent reversal of the mustard inhibition. If this is correct, then the normal rate of utilization of stroma lactate is slightly greater than we have estimated.

(3) Serine. Unfortunately we have but a single experiment directed toward a study of the possible transfer of serine from the stroma to the epithelium, and though this experiment yielded evidence of a strong accumulation of serine in the epithelium further confirmation is required.

The utilization of serine is inhibited by the same dose of mustard required to inhibit the utilization and transfer of lactate. If the mechanism for the utilization and transfer of lactate outlined above is correct, no special difficulties would be encountered in accounting similarly for the utilization and transfer of serine. Both the imine derived from the oxidation of serine and the deaminated product of this imine,—hydroxypyruvic acid,—would be titrated as serine in the analytic procedure used.

(4) Other substances. Butyrate injected into the stroma of the intact cornea is consumed at a rate similar to that for lactate and serine. The consumption of butyrate is inhibited by the same dose of mustard that inhibits utilization of lactate and of serine. We have no data on the possible utilization of butyrate by the denuded stroma, but, since oxidation would appear necessary for its utilization, consumption by the stroma would seem unlikely. If corneas are incubated without added substrate until the reserves of lactate are used up, alternate substrates are burned, and since there is no increase in non-protein nitrogen under these circumstances the alternate substrate are presumably fats. Consequently, the metabolic systems in the tissue which normally consume lactate can utilize fats as alternates. After exposure to mustard sufficient to inhibit the utilization of lactate, the

oxygen uptake drops in proportion to the reduced carbohydrate consumption. The alternates in this case cannot be utilized. It would appear, therefore, that butyrate might be a suitable example of an alternate to lactate and that its utilization is also carried out by the same mechanism.

Two substances of special interest, glutathione (8) and ascorbic acid (9) have been found present in the corneal epithelium in far higher concentration than in the stroma. No data are at present available to indicate whether these substances reach the epithelium from the stroma, from the tears, or are synthesized locally. Moreover, the existence of reversibly bound ascorbic acid has been demonstrated (10); consequently the high concentration of this substance in the epithelium need not indicate an inequality in free ascorbic acid between epithelium and stroma. Non-protein nitrogen is also found in higher concentrations in the epithelium than in the stroma.

III. *Loosening of the Epithelium.* The discussion in the preceding sections yields a picture of the boundary between stroma and epithelium as the seat of complex and intense metabolic activities. Some aspects of this metabolic activity are inhibited by mustard. The same dose of mustard which produces these metabolic effects also causes a loosening of the tissue cohesion at the stroma-epithelium boundary. We must now inquire whether any connections can be indicated between the metabolic phenomena and the pathological event of loss of cohesion.

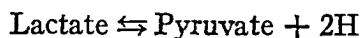
In the studies on the loosening of the epithelium (11) it was found that proteolytic enzymes on the one hand, and unionized surface active substances, such as butyl and amyl alcohol, on the other, disrupt the coherent boundary. These findings were taken to indicate that the cohesive boundary consists in part of a protein-lipoid multilayer. We can now suggest that the lipid component of this multilayer is responsible for the relative impermeability of the boundary to ions, and its consequent high electrical resistance.

EFFECT OF IODOACETATE AND FLUORIDE

The tissue cohesion is well maintained in the absence of oxygen and in the presence of cyanide and azide, but loosening of the epithelium occurs after application of iodoacetate and of fluoride. In view of this we have previously suggested that energy from glycolysis may be re-

quired for the maintenance of the cohesive system, and this is supported by the observation that partial loosening occurs after very prolonged incubation (24 hours) in the absence of added substrate, under which circumstances the available substrates for glycolytic break-down are largely exhausted. However, the matter is somewhat more complicated. Iodoacetate produces a very marked inhibition of glycolysis, and causes a loosening of the epithelium. The effect of iodoacetate might, therefore, be the result of an inhibition of glycolysis. With fluoride, which also inhibits glycolysis, a paradoxical situation arises. If fluoride is administered to the cornea in external bathing fluid, it causes a loosening of the epithelium if the tissue is incubated aerobically, but fails to do so under anaerobic incubation. However, when fluoride is injected into the stroma it causes a loosening of the epithelium both in the presence and in the absence of oxygen. Now when fluoride is administered in the external bathing fluid and the tissue is incubated anaerobically, a marked inhibition of glycolysis is observed. Under these experimental conditions the endogenous supply of glucose is rapidly exhausted in the controls without fluoride. Therefore, most of the excess lactate produced in the controls is derived from glycogen in the epithelium. It follows that fluoride supplied externally under anaerobic conditions is able to inhibit glycolysis in the epithelium, and, hence, that glycolysis, at least in the epithelium, is not necessary for the maintenance of the tissue cohesion. Consequently the loosening of the epithelium caused by iodoacetate cannot be attributed to inhibition of glycolysis, at least so far as the epithelium is concerned. The mechanism of loosening by iodoacetate is, therefore, independent of that by fluoride. Iodoacetate does not inhibit the utilization of stroma lactate. It differs from mustard both in this respect and also in the fact that the loosening is produced by iodoacetate both aerobically and anaerobically.

When fluoride is administered and the tissue incubated aerobically, it causes inhibition of glycolysis and also inhibition of utilization of stroma lactate, and the epithelium is loosened. It might be supposed that the failure in utilization of lactate under these circumstances is due to an inhibition of pyruvate oxidation displacing to the left the reaction:



However, the tissue can dispose of pyruvate by dismutation at a very rapid rate even in the presence of fluoride. Consequently, the effects of fluoride on lactate utilization must be on the mechanism that oxidizes lactate, unrelated to any effects that it may have on pyruvate oxidation in addition. We have here a parallelism between mustard and fluoride, both of which inhibit the utilization of stroma lactate and cause loosening of the epithelium. There is, however, an important difference. The loosening by mustard does not occur on anaerobic incubation, that by fluoride injected into the stroma does. Consequently the injury by fluoride is more directly disruptive of the cohesive boundary than that of mustard.

One further conclusion is to be drawn from the fluoride experiments. Under anaerobic conditions fluoride introduced into the stroma causes loosening of the epithelium, but when introduced into the epithelium it is without effect, and therefore, the stroma-epithelium boundary is impermeable to fluoride under anaerobic conditions. When the same experiment is performed aerobically loosening occurs no matter on which side of the boundary the fluoride is presented. It follows either that fluoride can loosen the epithelium by either of two independent mechanisms, or that the boundary between epithelium and stroma is less impermeable under aerobic than under anaerobic conditions. These comments suffice to show that the cohesive system is a highly complex organization, and has several points of vulnerability.

EFFECT OF MUSTARD

In spite of these obvious complexities we can, at least, hold fast to what little we do know about the effects of mustard, and inquire whether there is any possible connection between them. Mustard causes loosening of the tissue on aerobic but not on anaerobic incubation. Consequently, oxygen is necessary for the effect, and either some new oxidative channel is opened by mustard or some normally compensating reduction is blocked. The fact that the oxygen uptake of the tissue diminishes after exposure to mustard makes the first alternative unlikely. Normally the tissue consumes the stroma lactate, presumably by oxidation, and reasons have been given above indicating that this utilization of stroma lactate takes place at, or near, the stroma-epithelium boundary. When lactate is oxidized something else is

reduced. Mustard inhibits the utilization of stroma lactate, consequently at some point higher up in the affected hydrogen transport system something else fails to be reduced. We have, therefore, a potential link between the metabolic effects of mustard on lactate utilization and its effects on the cohesive boundary.

Under anaerobic incubation the loosening effect by mustard does not occur, and, in addition, some repair of the primary damage does occur, for subsequent aerobic incubation results in significantly less loosening of the epithelium than aerobic incubation without preliminary anaerobic incubation. There is a similar effect of a period of anaerobiosis following exposure to mustard in respect to the inhibition of lactate utilization. Moreover, the dosage range of mustard in respect to these two effects is identical. Finally, the effect on lactate utilization develops within two hours after exposure, while the loosening requires about six hours of incubation to become manifest. All this can be brought together in the supposition that the boundary itself contains components of the hydrogen transport system for utilization of the stroma lactate, and that oxidation of some of these components destroys some of the cohesive forces, perhaps by rendering these components susceptible to proteolytic cleavage.

NUCLEAR EFFECTS

In formulating an hypothesis as to the mechanism of mustard injury which leads to a loosening of the corneal epithelium, one can at least attempt to link the pathological phenomenon with those metabolic disturbances that have been found to be associated with it. Even such a tentative guide is lacking when we face the problem of the disturbances in nuclear physiology which result from exposure to mustard, for these disturbances occur at dosage levels at which no metabolic abnormalities have as yet been found. An attempt at an hypothesis regarding these effects can be guided only by analogy. The boundary between some tissues appears to be both the seat of active metabolic interaction and to contain components that react readily with mustard. Similar metabolic interactions and similar chemical reactivity may perhaps be found at the nuclear boundary.

IV. *Comment.* In the preceding discussion we have attempted to bring together the available information on the metabolic interactions

between epithelium and stroma, the effect of mustard on these interactions, and the effect of mustard on tissue cohesion. In order to illustrate one way in which this information might be combined, a series of unsupported hypotheses have been introduced, chiefly centering around the assumption that the metabolic interaction may be located at the boundary of cohesion. There are very serious reasons for believing that any such unifying theory is untrue, and this applies, a fortiori, to the particular hypotheses that have been suggested to illustrate how a unifying theory might be constructed. Each of the phenomena that we have sought to bring together is complex, and our knowledge of each is very incomplete. Moreover, the effects of mustard are themselves complex, and several different effects have been shown to be mutually independent. If our work in this field were to be continued, we should not have the temerity to suggest these unsupported hypotheses, but should merely use them as guides in the choice of further experiments.

The justification for formulating these hypotheses at the present time, however, is not merely to pass on to others our current working hypotheses, however small their value may be, but to show that the subject matter has actually reached a stage at which specific working hypotheses and a tentative general theory can be formulated in terms susceptible to experimental verifications or rejection. The gap in our knowledge between the biochemical and the pathological effects of mustard is still unbridged, but we can at last ask specific questions as to the possible construction of some specific bridges.

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APPENDIX I

THE TOLERANCE OF RABBIT CORNEA FOR VARIOUS CHEMICAL SUBSTANCES*

WILLIAM F. HUGHES, JR.,

In the course of the studies reported in the preceding papers a great number of substances were injected into the corneas of rabbits and the resulting reactions observed. These experiments were performed, in part, as controls for a wide variety of special studies, in part as a preliminary survey of the general toxicology of the corneal tissue. The results are compiled in the table below.

Unless otherwise noted, 0.1 ml. of the solution of test substance was injected intracorneally, using a #25-27 gauge needle and tuberculin syringe. Occasionally the anterior chamber was entered accidentally, in some cases resulting in a persistent edematous bulging of the cornea which could be identified clinically and the false positive corneal reaction discarded. The exact quantity injected within limits of 0.05 cc.-0.2 cc. was of less importance than the concentration of the injected material. Secondary infection was uncommon. Accidental injection of air into the cornea did not increase the severity of the reaction produced.

The reactions following intracorneal injection were similar in severity to those following mechanical removal of the corneal epithelium with a cotton toothpick swab, followed by irrigation for 10 minutes with the test solution.

A numerical grading of the severity of the ocular reaction follows the scheme outlined previously (1). The single numerical value represents the sum of the maximum values of each symptom observed over a period of 7-14 days, expressed in percentage of the maximum possible total.

Concentrations of solutions are expressed in terms of Molarity ("M") unless otherwise specified.

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* The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

TABLE I
Tolerance of Rabbit Cornea for Various Chemical Substances

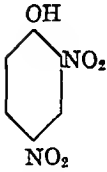
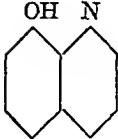

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Miscellaneous Agents</i> (Hypertonic, Dehydrating, Agents, Solvents, etc.)				
H ₂ O	0		0	All hypotonic solutions of non - toxic agents.
NaCl	0.9% (0.16M)		0	Isotonic
	2.3%	1	5	Hypertonic-small bullae produced
	4.5%	1	10	Large bullae
	9.0%	1	25	Edematous bulging of cornea
C ₂ H ₅ OH	50%	1	20	Non - progressive lesion
	3%	1	0	
C ₆ H ₅ OH (Phenol)	0.08M	1	10	
C ₆ H ₅ N (Pyridine)	0.08M			
	pH8	1	0	
	pH7	1	0	
 2,4 dinitrophenol	0.05M	1	52	
	0.012M	1	2	
	0.003M	1	5	
HCHO Formaldehyde	5.3M	1	70	
	0.33M	1	52	
	0.016M	1	37	
 8-hydroxy-quinoline	0.01M	1	5	
 (C ₂ H ₅) ₂ = N-C-SH diethyldithiocarbamate	0.05M	2	0	
	0.01M	2	0	

TABLE I—Continued

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Miscellaneous Agents—Continued</i>				
Methyl Glyoxal CHO C=O CH ₃	1% 0.25% 0.06% 0.015%	1 1 1 1	60 10 2 0	
Putrescine NH ₂ (CH ₂) ₄ ·NH ₂		2	16	
Taurine NH ₂ (CH ₂) ₂ SO ₃ H		2	15	No corneal reaction until 4 days after injection in one eye.
Cadaverine NH ₂ (CH ₂) ₆ ·NH ₂		2	1	
<i>Solvents</i>				
Propylene Glycol	5 min. irrigation with 100% P.G.	1	50	Hydroscopic agent
	5 min. irrigation with 50% P.G. in water	1	2	
	2 drops	10+	4	Conjunctival irritation only
	0.1 cc. intra-corneally	1	25	
Ethylene Glycol	2 drops	10	4	Conjunctival irritation only
Thiodiglycol	5 min. irrigation	1	62	
	1 min. irrigation	1	25	
	2 drops	5	4	
	0.1 cc. intra-corneally	4	45	

TABLE I—Continued

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Solvents—Continued</i>				
Isopropyl Alcohol	3 min. irrigation with 50% Isopropyl alcohol in water	1	45	
Aerosol	1 min. irrigation with 8% solution	1	32	
	4 min. irrigation with 1% solution	1	32	
	0.1 cc. of 1% intracorneally	1	50	
Triacetin	6 min. irrigation	1	0	Non - hydroscopic
	0.1 cc. intracorneally	1	25	
<i>Protein - Precipitating Agents (Anions)</i>				
Hydrochloric Acid or Trichloroacetic Acid	<pH 3 >pH 3		+ 0	
"Universal Buffer" (citrate-phosphate-borate)	<pH 4.5 >pH 4.5		+ 0	
Metaphosphoric Acid or Sulfosalicylic Acid	<pH 5.5 >pH 6.0		+ 0	
Picric acid or Tungstic acid or Tannic acid	pH 7-9		+	
<i>Mucoid - Dissolving Agents (Alkalies)</i>				
NaOH	>pH 11.5 <pH 11.5		+ 0	
Ca(OH) ₂	0.014M (Sat. Soln.)	1	52+	

TABLE I—*Continued*

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Metal Ions</i>				
CaCl ₂ (neutral solution)	5.4M sat. soln.	1	75	Hypertonic
	1.0M	1	50	Hypertonic
	0.08M	6	2	Intracorneal injections
	0.08M	5	45	Mechanical removal of corneal epithelium followed by 10 min. irrigation
BaCl ₂	0.08M	2	0	At pH5 or 7.
	0.08M	10	50	Intracorneal Mechanical removal of epithelium followed by 10 min. irrigation.
MgSO ₄	0.08M	1	0	
FeCl ₃	0.05M	1	32	
	0.003M	1	32	
	0.0015M	2	4	
Pb(Ac) ₂	0.08M	2	100	Same reactions produced at pH 4.2 and pH 6.0
	0.1–0.01M	3	50	
	0.003M	2	35	
	0.0012	1	7	
	0.0006	1	0	
	0.0003	1	0	
ZnCl ₂	0.08M	1	40	
	0.0025M	2	25	
	0.0013M	1	35	
	0.0006M	1	38	

TABLE I—Continued

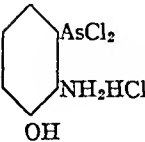
SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES	
<i>Metal Ions—Continued</i>					
<i>ZnCl₂</i>	0.0003M to 0.00004M	4	0	pH 5.0 pH 5.5	
<i>CuCl₂</i>	0.16M 0.08M	1 1	72 65		
<i>CuSO₄</i>	0.008–0.002M 0.001–0.0005M	3 3	30 17		
<i>HgCl₂</i>	0.08M 0.001M 0.006M 0.003M 0.00015M to 0.00002M	1 1 2 2 4	75 60 22 8 0		
<i>Arsenicals</i>					
<i>ClCH=CH–AsCl₂</i> (Lewisite)	0.01Mgm		75		Calculated from analysis of arsenic intracorneally
<i>C₆H₅AsO</i> Phenylarsine oxide	0.01M	1	100		
 <i>AsCl₂</i> <i>Mapharsen</i> <i>NH₂HCl</i> <i>OH</i>	0.04M 0.004M	1 1	85 40		
<i>NaAsO₂</i> Sodium meta-arsenite	0.01– 0.005M 0.0008M 0.0004M 0.0002M 0.0001M	2 1 1 1 1	87 22 15 5 0		
<i>Na₂AsO₄</i> Sodium arsenate	0.08M	4	75		

TABLE I—Continued

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Arsenicals—Continued</i>				
$\begin{array}{c} \text{NH}_2-\text{C}=\text{O} \\ \\ \text{CH}_2 \\ \\ \text{NH Tryparsamide} \\ \text{Cyclohexyl} \\ \\ \text{HO}-\text{As}-\text{ONa} \\ \\ \text{O} \end{array}$	0.04M 0.004M	1 1	0 0	
Sodium selenite	0.05M 0.012M 0.003M	1 1 1	97 82 77	
<i>Oxidizing Agents</i>				
H_2O_2	0.3% 0.25% 0.12% 0.10% 0.06% 0.03% 0.01% 0.003% 0.001%	1 1 1 1 1 1 1 1 1	60 95 62 50 50 45 30 37 0	Intracorneally Irrigation
KMnO_4	0.08M 0.028M 0.009M 0.003M 0.001M	1 1 1 1 1	60 35 50 0 0	Intracorneally Irrigation
+++ $\text{K}_4\text{Fe}(\text{CN})_6$	0.07M	2	5	
I_2 in 3% KI	0.1M 0.02M 0.01M 0.005M	1 2 1 1	95 30 15 0	
KI alone	3% 1%	1 1	17 0	
KIO_3	0.16M 0.03M	1 2	22 0	pH 5 and pH 7.0

TABLE I—Continued

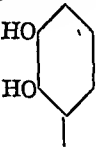
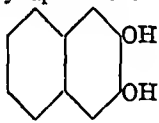
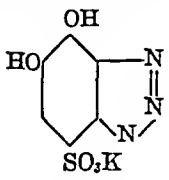
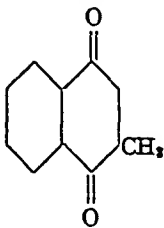
SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Oxidizing Agents—Continued</i>				
NaClO_4 (Perchlorate)	0.08M	2	3	
NaClO_2 (Chlorate)	0.08M	1	20	
$\text{Na}_2\text{Cr}_2\text{O}_7$ (dichromate)	0.08M	1	70	
Na_2CrO_4 (chromate)	0.08M	1	75	
<i>Quinones and Hydroquinones</i>				
3-Allyl-catechol	0.05M	1	45	
	0.025M	1	12	
	0.012M to 0.0015M	4	0	
Dihydroxynaphthalene	0.05M	2	42	
				
Potassium - benzotriazole - hydroquinone sulfonate	0.05M	2	37	
				
2 Methyl 1,4 naphthoquinone (Vitamine K)	0.05M	2	38	
	0.025M	4	18	

TABLE I—Continued

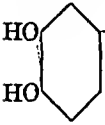
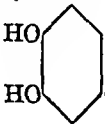
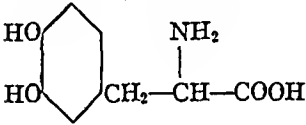
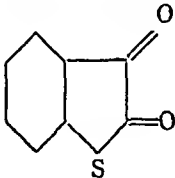

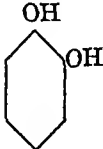
SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Quinones and Hydroquinones—Continued</i>				
Adrenalin	0.05M	1	17	
 <chem>OC1=CC=C(C=C1)C(O)CN</chem>	0.025M	2	12	
Vit. K ₃ Adrenalin	0.025M of each	2	35	
4-Allyl-catechol	0.05M	1	37	
 <chem>Oc1ccc(O)cc1C=CC</chem>	0.025M	1	30	
	0.012–0.003M	3	5	
	0.015M	1	0	
Dihydroxyphenylalanine ("dopa")	0.05M	2	22	
 <chem>N[C@@H](Cc1c(O)cc(O)cc1)C(=O)O</chem>				
Thionaphthenequinone	0.05M	2	12	
 <chem>O=C1C(=O)C2=CC=CC=C2S1</chem>				
p-hydroquinone	0.05–0.012M	3	5	
 <chem>Oc1ccc(O)cc1</chem>				
catechol	0.05–0.012M	3	0	
 <chem>Oc1ccccc1O</chem>				

TABLE 1—Continued

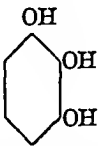
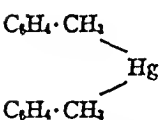
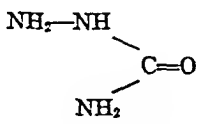
SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES
<i>Reducing Agents</i>				
Na ₂ S ₂ O ₃ (thiosulfate or "hypo-sulfite")	0.08M	2	15	
	0.02-0.01M	2	2	
	0.008-0.005M	3	5	
	0.0025-0.0006M	3	2	
NaHSO ₃ (bisulfate)	0.08M	1	7	
	0.008M	1	0	
NaNO ₂ (nitrite)	0.08M	1	0	
 (Pyrogallol)	0.08M	1	10	
	0.008M	1	7	
<i>Sulphydryl-Binding Agents</i>				
ICH ₂ COOH (Iodoacetate)	0.1M	1	100	
	0.001M	1	70	
C ₆ H ₅ ·HgOH (Phenyl-mercuric hydroxide)	0.1M	1	77	
	0.001M	1	25	
C ₆ H ₅ ·HgCl	0.1M	4	66	
	0.01M	1	47	
	0.001M	1	37	
	0.01M	1	0	
	0.001M	1	0	
<i>Miscellaneous Inhibiting Agents</i>				
NaCN (cyanide)	0.1M	3	46	pH 6 and pH 7
	0.001M	1	10	
NaN ₃ (azide)	0.1M	3	2	
	0.001M	1	5	
 (semicarbazide)	0.1M	1	0	
	0.001M	1	0	

TABLE I—Continued

SUBSTANCE	CONCENTRATION	NO. EYES TESTED	SEVERITY OF REACTION	NOTES	
<i>Miscellaneous Inhibiting Agents</i> —Continued					
$\begin{array}{c} \text{NH}_2 \\ \diagdown \\ \text{C}=\text{S} \\ \diagup \\ \text{NH}_2 \end{array}$ (thiourea)	0.08M	1	22	Late reaction	
$\begin{array}{c} \text{CH}_2-\text{CH}-\text{CH} \\ \\ \text{NH} \\ \\ \text{NH}_2-\text{C}=\text{S} \end{array}$ (Allylthiourea)	0.05M	2	18	Moderate conjunctival edema up to 3 hours after injection	
	0.01M	2	6		
NaF (fluoride)	0.02M	1	25		
	0.01M	1	15		
	0.005–0.006M	4	0		
Histamine	1:10,000	2	0		
	1:100,000	1	0		
	1:1,000,000	1	0		
Pontocaine	0.5%	1	42		
Eserine	0.25%	1	2		
<i>Dyes</i>					
Crystal Violet (phenylmethene Rosaniline dye)	0.5%	1	57	pH 7.0 or pH 3.5	
	0.06%	1	70		
	0.007	1	15		
Phenosafranin (azin dye)	0.5%	1	67		
	0.06%	1	70		
	0.007%	1	0		
Acriflavine (xanthene - acridine dye)	0.1%	2	42		
	0.05%	1	45		
	0.01%	1	45		
Brom Phenol Blue (xanthene-sulphonphthalein dye)	0.05%	1	32		

TABLE I—*Concluded*

SURFACE	CONCENTRATION	NUMBER OF EYES TESTED	SEVERITY OF REACTION	NOTES
Methylene Blue (Thiazine dye)	0.5%	1	35	
	0.06%	1	37	
	0.007%	1	0	
Malachite Green (phenyl- methane dye)	0.05%	1	35	
Toluidine Blue (thiazine dye)	0.5%	2	28	
	0.1%	1	7	
	0.05%	2	11	
Methyl Green (Rosanilin dye)	0.05%	1	15	
Fluorescein (xanthene dye)	0.05%	1	6	
Congo Red (Azo dye)	0.5%	1	9	
	0.06%	1	12	
	0.007%	1	0	
Pyronin (xanthene dye)	0.05%	1	0	
Thionine (thiazin dye)	0.05%	1	0	
Rose Bengale (xanthene dye)	0.05%	1	0	

APPENDIX II

A MECHANICAL DEVICE FOR THE EXTRACTION OF SOLUBLE COMPOUNDS FROM THE CORNEA AND OTHER TOUGH TISSUES*

JONAS S. FRIEDENWALD AND SYLVIA G. MOSES

Biochemical studies on the cornea, skin, and other tough tissues are often handicapped by the amount of manual labor involved in grinding the tissue. Thus, in determining the acid soluble, non-protein nitrogen of the cornea, it was necessary to grind each sample in a mortar with the extracting fluid for 15 minutes. None of the available tissue grinders or homogenizers (1) are suitable for extraction of such tissues. However, the mechanical device illustrated in the figures was found to yield equally satisfactory extracts. It consists of a vessel (Figs. 1, 2) at the bottom of which is a pit suitable to contain the tissue sample which is to be extracted. A plunger, the tip of which fits loosely into this pit, is used to squeeze the tissue. Above the tip of the plunger is a flange which fits loosely into the main cylindrical compartment of the vessel and prevents the extracting fluid from splashing when the plunger is lowered. Three grooves are cut in the margin of the flange to allow air or fluid to pass the flange as the plunger is raised or lowered. The plunger is moved slowly up and down by a lever. With a lever ratio of 10, a weight of 2 lbs. on the end of the lever was found convenient, and 200 strokes of the weighted lever sufficient to produce as complete an extraction as 15 minutes grinding in a mortar, in each case with two washings of the tissue.

If single samples are to be extracted, the lever may be operated manually. For larger scale operation, six vessels were mounted in a frame, each under its plunger and lever. The plungers were raised and lowered by a set of eccentric wheels attached to a shaft turned at a rate of one revolution every 4 seconds by a $\frac{1}{80}$ HP motor with an appropriate reduction gear.

* The work described in this paper was done in largest part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Johns Hopkins University.

Our use of this device has been concerned chiefly with non-protein nitrogen determination in trichloroacetic acid extraction. For this purpose vessels and plungers were machined from a 2 inch Lucite rod. The

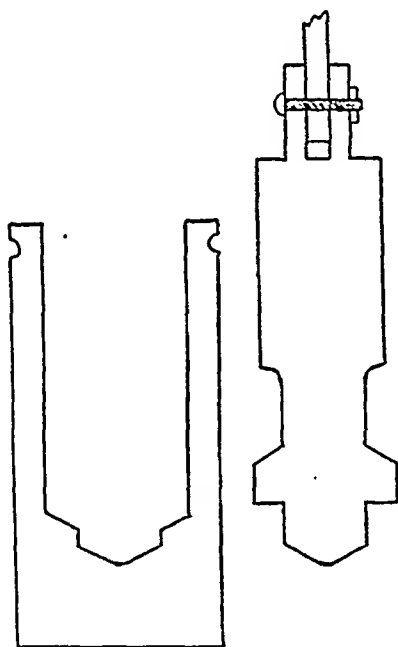


FIG. 1

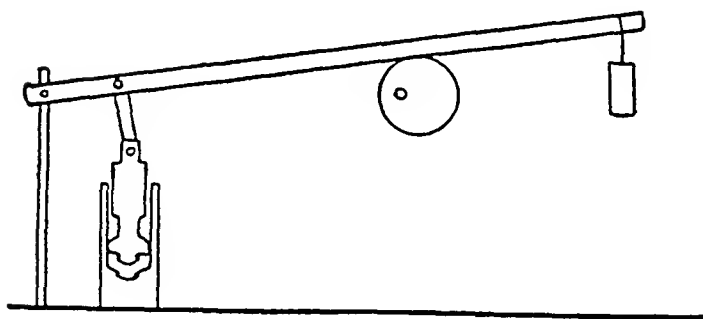


FIG. 2

procedure arbitrarily adopted for convenience in using the mechanical extractor consists of a ten minute extraction followed by two five minute washings. After using this device for several hundred samples, we have found it completely satisfactory. It might be mentioned that

we were surprised to find that the simple process of squeezing in this manner gave as good results as the squeezing plus shearing of manual manipulation in a mortar.

REFERENCES

- 1) UMBREIT, W. W., BURRIS, R. H., AND STAUFFER, J. F.: Manometric Technique and Related Methods for the Study of Tissue Metabolism. Burgess Publ. Co., Minn. 1945.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

Nurse-Patient Relationships in Psychiatry. By HELENA WILLIS RENDER. Illus. 346 pp. \$3.00. McGraw-Hill Book Company, Inc., New York, New York' 1947.

Mrs. Render has made an outstanding contribution to the field of psychiatric nursing. Her book is instructive and direct, yet detailed and lucid. Her suggestions come from a keen and warm understanding of the needs of patients and are practical and sound. She has shown the field of psychiatric nursing to be worthy of the sincere efforts of a mature and understanding person, and has revealed the dignity, satisfaction, and challenge in this field of nursing. This is an excellent book and might be read with profit by both doctors and nurses.

A. B.

Nursing Care in Chronic Diseases. By EDITH L. MARSH. Illus. 237 pp. \$3.25. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1946.

The objective of this book, as stated in the foreword, is "to promote for the greatly neglected chronic invalid a high degree of enlightened care and human understanding."

The subject matter includes a review of home and hospital facilities available for the chronically ill and a frank, unbiased discussion of present day inadequacies in care. As the various chronic conditions are presented for study, one is impressed by Miss Marsh's broad sociological viewpoint and her unmistakable interest in the patient as a sick person in need of more and better nursing care. She emphasizes the value of cheerful, sympathetic, humane care, and of treating each patient as a rehabilitation problem even though the result may be only of a temporary nature. Miss Marsh has made this a truly inspirational and practical book which should be helpful to students and faculties in schools of nursing and to the staffs of social and public health agencies.

V. B.

Professional Adjustments. By SISTER MARY ISIDORE LENNON. 299 pp. \$3.00. The C. V. Mosby Company, St. Louis, Missouri, 1946.

In this book Sister Mary Isidore Lennon has endeavored to set forth in simple, comprehensive form material which should enable the beginning student in a school of nursing to achieve happiness in her service of the sick, in her studies, and in her social life. She writes with a real understanding of the difficulties confronting the very young nursing student of today who, in most of our hospitals, is expected overnight to cast aside the carefree ways of adolescence and to acquire the maturity which should characterize the professional woman.

The author writes primarily for students in Catholic hospitals and emphasizes the Catholic viewpoint, which limits somewhat the usefulness of the book. It probably would not be acceptable as a text for non-Catholic students, although the practical suggestions offered along the lines of character development and ethical practice in nursing would undoubtedly be of interest to lay instructors in schools of nursing.

V. B.

Retinal Structure and Colour Vision. By E. N. WILLMER. Illus. 431 pp. \$4.50. The Macmillan Company, New York, New York, 1947.

Under the new assumption that the rods, comprising 95% of the visual receptors, are not blind in daylight, the author boldly discusses the possibility that rods and cones contribute equally to the effects of color mixture: rods giving blue-greens and cones red-yellow. Forced, however, by the evidence, to three variables, he postulates the "day-rod", whose resultant color sensitivity is that of rhodopsin minus iodopsin, achieved by neural inhibition. Poliak provides a structural basis for this.

Present data, at the level of bipolar and ganglion cells, insufficiently support the author's attempt to explain by this hypothesis the usual test cases of color phenomena. Consequently his quantitative discussion is quite unconvincing. Adaptation and brightness are particularly unsatisfactory. His main contribution is the demonstration that known neural behavior suffices qualitatively to account for chromatic phenomena, without postulating the unobserved photopigments in cones required by current color theories. The use of recent data from Granit, Hartline and Poliak lends the argument a force and verisimilitude denied to E. Q. Adams' more adequate theory of bipolar inhibition (1924). Willmer's striking observations on the blue-blindness of the fovea are still controversial quantitatively; so that the assignment of blue response to rod function, though now possible on grounds of retinal structure, is yet unproved.

Excellent illustrations, several in color, lend interest to a rather technical work, intended for readers familiar with the complexities of color vision.

S. A. T.

Principles and Practice of Medicine, The. (Originally written by William Osler.) By HENRY A. CHRISTIAN. 16th ed. 1539 pp. \$10.00. D. Appleton-Century Company, Inc., New York, New York, 1947.

The original text of this book was written by Osler and published in 1892. During the past nine years, under the editorship of Henry A. Christian, it has undergone radical changes and modernization. There is still much of the original manuscript, but one is frequently aware of the present author's literary style.

In this edition, Dr. James G. Carr, in a short preliminary paragraph, traces the history of medicine (1892-1947) as told in sixteen editions of Osler's "Practice and Principles of Medicine." It is really quite interesting to follow the changes in the importance of various diseases, the emphasis on physiological abnormality instead of gross and microscopic pathology, and the revolutionary change from therapeutic

nihilism to the profuse renaissance of present day therapeutic enthusiasm. It is heartening to consider the many advances in the principles and practice of medicine that these sixteen editions of Osler's textbook record.

In this edition, Dr. Christian genuflects very slightly to psychosomatic medicine. He points out:

"In latter years, with the enormous development of laboratory procedures and the increased utilization of complex techniques of examining the patient, the physician has found it increasingly easy to neglect the patient's personality and surroundings in his management of him, with a resultant feeling on the part of the patient that he is missing something that is traditional of the general practitioner that served his forebearers. As a resultant, renewed emphasis is coming to be placed again on the emotional and functional aspects of disease, with an increased introduction into our hospitals of psychoneurologists to turn the minds of the students and internes toward functional disease and functional aspects of organic disease in an effort, as yet not too satisfactory, to rebridge a recognized dilemma in modern medicine with a point of view expressed in the terms, Psychosomatic Disease and Psychosomatic Medicine. Often this is looked upon as something new. Its newness, however, chiefly is in the terms now used for what the former generations of medical men practiced without much use of names for it."

This edition, however, opens "with a discussion of some of the diseased conditions now recognized as essentially non-organic in nature, in the hope that from reading a discussion of them the student and practitioner will obtain knowledge toward a more satisfactory understanding of what ordinarily is looked upon as purely organic disease. It is to be recognized that every patient with organic disease presents a functional, emotional reaction which varies with the personality, the economic and social relationship, and the race and heredity of the individual, and that his problem as a patient can be solved only by integrating his functional and somatic disabilities after a thorough study of both."

The book is up to date in its detailed description of penicillin therapy, of streptomycin therapy, of the part that sulfonamides play. One is interested to see how in a book of some 1374 pages endocrine diseases are accounted for in 40 pages while the same space is devoted to pneumonia, and typhoid fever fills 30 pages. A feature which Dr. Christian introduced several editions ago is the "References" at the end of each chapter. In the references are usually one or two of the early classic papers and a few of the outstanding recent ones. This allows the student to pursue any subject further.

Some of us who became very well acquainted with tropical diseases may regret that Dr. Christian has not made more of an effort to bring these subjects up to date. However, one must confess that we see very few of these diseases that our armies have brought back with them from the tropics.

This is an excellent book. Dr. Christian has performed a brilliant task, and those of us with a sentimental feeling for the old Osler "Textbook" owe him a debt of gratitude.

H. M. T., Jr.

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RELATION OF DOSAGE SCHEDULE TO THERAPEUTIC EFFICIENCY OF STREPTOMYCIN IN THE TREATMENT OF *K. PNEUMONIAE* INFECTIONS IN MICE¹

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Keefer (1) states that in the treatment of infections in man with streptomycin it is necessary to give intramuscular injections every 3-4 hours. Paine, Murray and Finland (2) conclude that 1.0 g. of streptomycin every six hours is adequate for most infections. There is no experimental evidence to indicate that such frequent administration is essential for the optimal antibacterial efficiency of streptomycin. In several different infections in mice, it has been possible to obtain a high survival rate with a single intramuscular dose (3, 4, 5), or with two doses (6) of streptomycin. Single doses of 0.3 g. of streptomycin have been shown to cure gonorrheal urethritis (7).

Schedules of frequent administration of streptomycin in patients have been based possibly on analogy with the sulfonamides, with which drugs it is essential to maintain a blood concentration in order to obtain optimum effectiveness (8). It has recently been found (9) that aqueous penicillin G, in the treatment of hemolytic streptococcus infections in mice, is not demonstrably different in effectiveness whether given at twenty-four hour intervals or at one hour intervals. In other words, the antibacterial effect of penicillin G outlasts its presence in the mouse by many hours. Similar experiments were therefore undertaken to study the comparative effectiveness of streptomycin when administered at frequent and infrequent intervals to mice with a *K. pneumoniae* infection. The recent development of a chemical method (10) for streptomycin determination afforded an opportunity for a parallel study of the rate of disappearance of streptomycin from the blood of the same strain of mice.

¹ This investigation has been aided by a grant from the U. S. Public Health Service.

² Roche Fellow in Medicine and Experimental Therapeutics.

METHODS AND MATERIALS

1. *Mice.* The mice used in these experiments were albino mice of the Swiss strain, of uniform stock, from Tumblebrook Farm. The mice used in the infections weighed from 13–17 g., and those used in the plasma concentration studies weighed 18–21 g. For the infections

TABLE 1

Comparative therapeutic efficiency of streptomycin given intramuscularly in single and multiple doses to mice infected with K. pneumoniae

Mice: Tumblebrook Farm, albino, Swiss Strain, 13–17 g.

Organism: *K. pneumoniae*, Type B, Strain BE.

Infection: 0.5 ml. of 10^{-5} dilution of a 5 hour subculture, containing 7000 organisms.

Treatment: Streptomycin sulfate, intramuscularly, four hours after infection.

TOTAL AMOUNT OF STREPTOMYCIN BASE ADMINISTERED	PROPORTION OF SURVIVORS					
	Schedule					
	1	2	3†	4*	5†	6
mg./k.	Un- treated	Single Dose	3 Doses at 24 Hour Intervals	3 Doses at 8 Hour Intervals	3 Doses at 8 Hour Intervals	6 Doses at 4 Hour Intervals
64	0/50	15/20	17/20		20/20	20/20
32		14/30	16/20	16/20	15/20	18/20
16		17/30	11/20	13/20	33/40	16/20
8		7/30	4/20	13/20	18/40	4/20
4		5/30	0/20	3/20	1/40	1/20
2		9/30	0/20	5/20	0/20	0/20
1		0/30			0/20	
$\frac{1}{2}$		0/30			0/20	
SD50 mg./k.		22.0	15.4	8.3	9.5	11.0
Standard Error \pm mg./k.		4.8	2.1	2.4	0.8	1.3

* $\frac{1}{2}$ the total dose given in first dose, remainder in $\frac{1}{4}$ – $\frac{1}{4}$ fractions.

† Three equal doses.

three separate experiments were run, and, since the results were highly reproducible, they have been pooled (Table 1). The first experiment was a small one, designed to determine the dosage range for subsequent study. Groups of ten mice were infected and treated as an experimental unit, each unit receiving a single dose of streptomycin. In the second and third experiments twenty mice were used as a treatment unit for each variation in either the amount or timing of

streptomycin dosage. In the second experiment a single dose was compared to three doses at eight hour intervals, at eight different dosage levels. In the third experiment, schedules 3, 4, and 6 (Table 1) were compared to three doses at eight hour intervals. In each experiment the first and last groups of mice infected were held as untreated controls.

2. *Klebsiella Pneumoniae Infection.* The mice were infected with *K. pneumoniae*, Type B, Strain BE. Stock cultures were grown in trypticase-soy-phosphate rabbit blood broth, and were kept at 6°C. Virulence was maintained by frequent mouse passage. Virulence studies showed that 700 organisms (0.5 ml. of a 10^{-6} dilution) injected intraperitoneally killed all but an occasional mouse. Ten to a hundred organisms killed about half the mice. For the experimental work, mice were injected intraperitoneally with 0.5 ml. of a 10^{-5} dilution of a five hour blood broth culture. This inoculum averaged 7000 organisms and killed all untreated control mice. Deaths of 85 per cent of the controls occurred in 10-24 hours. The remainder died in 2-10 days. Cultures of heart blood from the two controls dying on the 7th and 10th days, respectively, yielded *K. pneumoniae*. Similar cultures of one-third of the treated mice dying after day 10 were made and also grew out *K. pneumoniae*.

3. *Streptomycin.* A single lot (Pfizer-P4718) of amorphous streptomycin sulfate was used for both the treatment and plasma concentration studies. Chemical assay by the method of Marshall, Blanchard and Buhle (10) showed that 77 per cent of this material was streptomycin base. Ninety-five per cent of the base was streptomycin A, as shown by counter-current distribution studies (11). Dosage was calculated in terms of streptomycin base per kilogram of mouse. Injections were made intramuscularly in 0.2 ml. volume, four hours after infection. Only those mice still alive 21 days after the end of treatment were counted as survivors.

At any given dosage level, the mice in the different dosage schedules received the same total dose of streptomycin base.

4. *Plasma Concentration of Streptomycin in Mice.* A large number of mice were injected intramuscularly with 50 mg./k. of streptomycin sulfate in saline solution containing 5 mg. of the base per ml. Groups of mice were bled at 15, 30, 60 and 120 minutes after injection, 0.25 ml.

of whole blood being obtained from each mouse by cardiac puncture. If less than 0.25 ml. was obtained, the sample was discarded and the next mouse bled. Blood from ten or more mice for each time interval was pooled in a centrifuge tube containing one drop of 30 per cent sodium oxalate per 3.0 ml. of whole blood. The tube was centrifuged

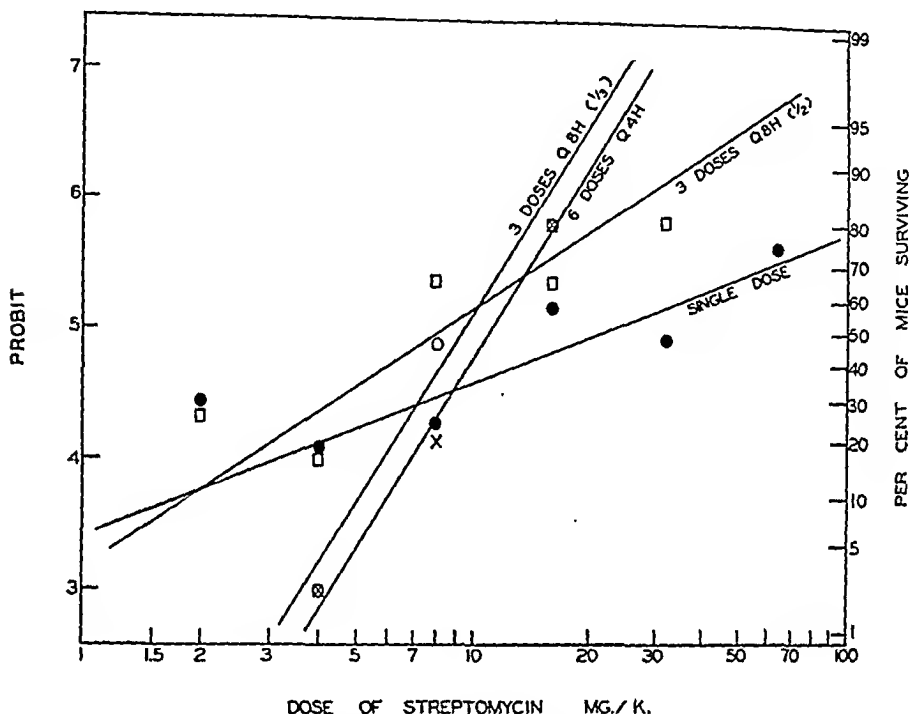


FIG. 1. Effect of intramuscular streptomycin upon survival rate of mice infected with *K. pneumoniae* (data taken from Table 1.)

- single doses.
- 3 equal doses given at 8 hour intervals.
- ×—× 6 equal doses given at 4 hour intervals.
- 3 doses at 8 hour intervals, given in $\frac{1}{2}$ — $\frac{1}{4}$ — $\frac{1}{4}$ fractions.

immediately, the plasma pipetted off, and the concentration of streptomycin determined by the method of Marshall, Blanchard and Buhle (10).

RESULTS

The results of the three experiments with *K. pneumoniae* infections are pooled in Table 1. The method of Litchfield and Fertig (12) has

been utilized for the calculation of the Median Survival Dose (SD50) and the standard error of the SD50.

1. *Effect of a Single Dose of Streptomycin.* When the *K. pneumoniae*-infected mice were given the entire amount of streptomycin in a single dose, erratic results are obtained (Table 1). Low doses

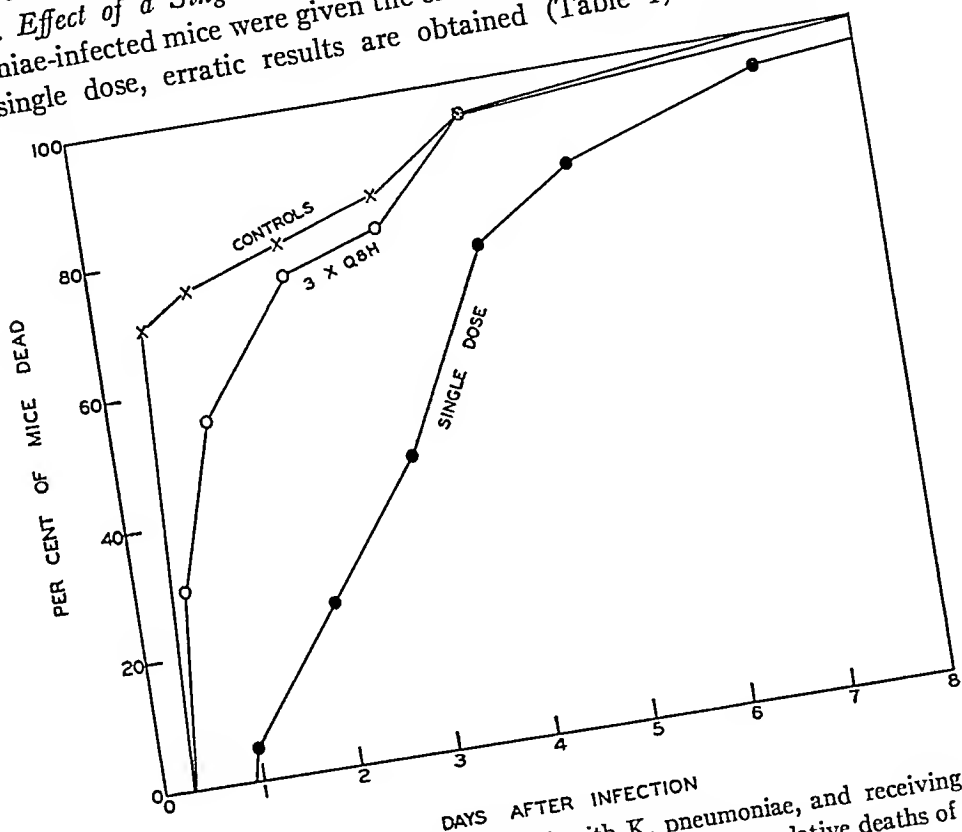


FIG. 2. Time of death of mice infected with *K. pneumoniae*, and receiving 1 mg./k. of streptomycin I.M. Each curve represents the cumulative deaths of 20 mice.

- — single dose.
- — 3 equal doses given at 8 hour intervals.
- × — untreated mice.

saved significant numbers of mice, but even the largest doses tried failed to save more than 75 per cent of the mice. For this reason Median Survival Doses do not form an adequate basis for comparison of this dosage schedule with those which give 100 per cent survival, such as 5 and 6 of Table 1.

In Figure 2 is shown the effect of a single dose of 1 mg./k. of streptomycin upon the length of survival of the mice. This is compared to the effect of the same total dose split into three fractions; given at eight hour intervals. It can be seen that even though all the mice

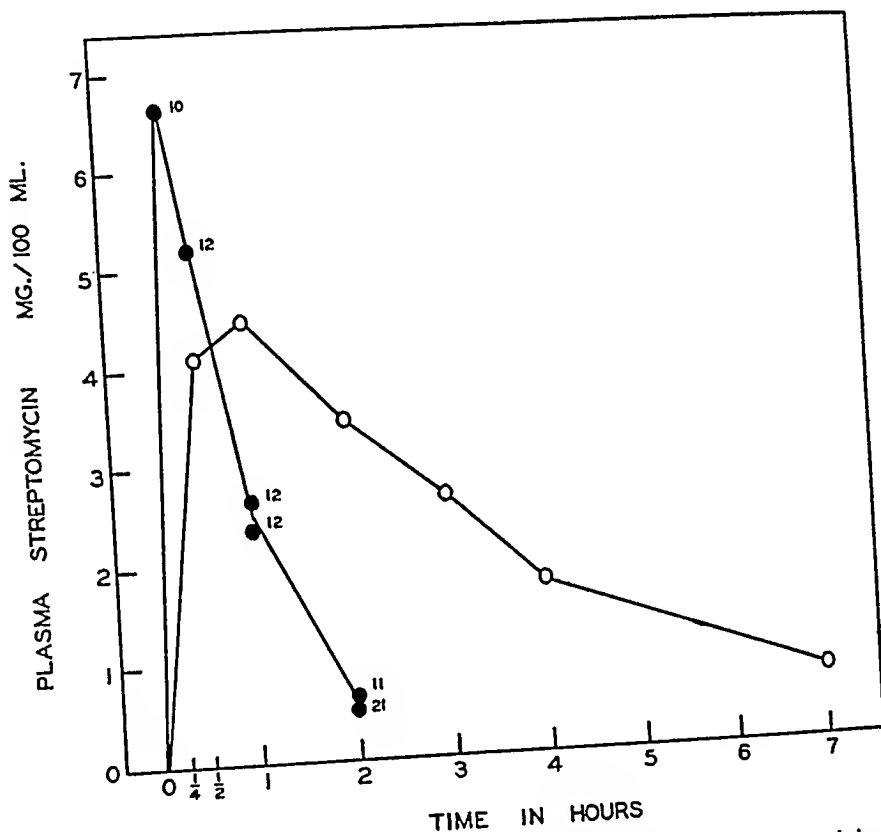


FIG. 3. Plasma concentrations of streptomycin in mice ●—● receiving 50 mg./k., and man ○—○ receiving 20 mg./k. in a single intramuscular dose. The numbers to the right of the solid circles represent the number of mice used for the determination.

ultimately die, longer protection was secured from the single dose than from the multiple doses.

2. *Effect of Multiple Doses of Streptomycin upon Therapeutic Efficiency.* When streptomycin is given in the treatment of *K. pneumoniae*-infected mice, there is no significant difference between a regimen of three doses at eight hour intervals and six doses at four

hour intervals (Table 1 and Figure 1). If the drug is given once every 24 hours for three doses, slightly larger amounts are required for the SD50. It is interesting to note in Figure 1 that dosage schedule 4 (three doses at eight hour intervals, with one-half the total amount given in the first dose) gives a dosage response curve intermediate in position between the single and other multiple dose curves. The "priming" dose given in the beginning apparently carries with it some of the characteristics of a single dose.

3. *Plasma Concentration of Streptomycin in Mice after Intramuscular Administration.* These are shown in Figure 3, together with the plasma concentrations obtained in man after a single intramuscular dose. Despite the fact that the mice, on a weight basis, received two and one-half times the quantity of drug given to man, plasma concentrations of above 0.5 mg. per cent are maintained in the mice for one-fourth as long as in man.

DISCUSSION

The dosage schedule (six doses at intervals of four hours), which most nearly approximates a constant plasma concentration in mice, is not significantly better than schedules of less frequency (three doses at eight hour intervals), and is only slightly superior to three doses at 24 hour intervals. Many other dosage schedules, particularly those involving more frequent administration, require testing before selection can be made of the maximally efficient therapeutic regimen for streptomycin. Nevertheless, from the data available, it is apparent that frequent dosage is not essential for the efficient utilization of streptomycin in *K. pneumoniae* infections in mice. In this respect, streptomycin resembles penicillins G and K (9) rather than the sulfonamides (8).

The comparative plasma concentration of streptomycin, after single intramuscular doses in mouse and man, indicates that the entire cycle of absorption of streptomycin from muscle, and disappearance from the plasma, is much more rapid in the mouse—two hours of equivalent plasma concentration in the mouse corresponding to about eight hours or more in man.

These facts and conclusions about the antibacterial effect of streptomycin in *K. pneumoniae* infections in mice are not necessarily

transferrable to the treatment of human infections. However, these experiments indicate that it is probably unnecessary to administer streptomycin to man in such a fashion as to maintain constant plasma concentrations. On that basis alone, because of less pain and more rest for the patient, schedules of less frequent administration are worthy of trial in human infections. When, in addition, one considers that a constant plasma concentration of streptomycin has been associated with the rapid emergence of resistant organisms, and with considerable human toxicity, further need for such trial becomes apparent. It is important to point out that trials of infrequent administration of streptomycin should be undertaken in carefully selected cases and under close observation. Specific suggestions for dosage regimens in streptomycin-susceptible infections in man cannot be extensive as each case presents its own problem. However, it seems justifiable to proceed with a dose of 1.0–2.0 g. intramuscularly every 12 or 24 hours, in acute infections, until the temperature is normal. In fulminating infections, it is probable that small fractions of streptomycin given at frequent intervals may fail to control the large bacterial population. Hence it is suggested that a "priming" dose of 2.0 g. be given such patients as soon as possible. Larger single doses should not be given unless it can be shown that toxicity is a function of long continued constant plasma concentration, rather than of peak concentrations.

SUMMARY

1. In the treatment of mice with *K. pneumoniae* peritonitis with intramuscular streptomycin, there is no significant difference in results between schedules of administration at four hour intervals or at eight hour intervals.

2. When single doses of streptomycin were used, large increments in dosage gave only small changes in survival percentages of the mice.

3. Mouse plasma streptomycin concentrations remained above 0.5 mg. per cent for two hours after a single intramuscular dose of 50 mg./k. In man, after a single dose of 20 mg./k. plasma concentration remained above 0.5 mg. per cent for seven hours.

4. The implications of these experimental findings in the treatment of streptomycin-susceptible infections in man have been discussed.

The author wishes to thank Dr. E. K. Marshall, Jr. for helpful advice; Dr. Harold J. White, American Cyanamid Co., for the K. pneumoniae; Dr. Oskar Wintersteiner, of the Squibb Institute for Medical Research, for the counter current distribution studies; Dr. R. A. Patel-ski, of Chas. Pfizer and Co., for the streptomycin sulfate; and Mrs. Evelyn Epperson for technical assistance.

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STUDIES ON SCHISTOSOMIASIS JAPONICA IN THE PHILIPPINE ISLANDS

3. A CLINICAL STUDY OF 72 CASES TREATED WITH TARTAR EMETIC

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The epidemic of Schistosomiasis japonica which occurred on Leyte, Philippine Islands, started in November 1944, reached its peak in January and died out in May of 1945. Billings, Winkenwerder and Hunninen (1) reported on the signs and symptoms of the acute stage. A second report by Winkenwerder et al (2) is an analysis of the effect of treatment with 40 cc. of Fuadin. The present report concerns 72 patients treated with tartar emetic, 32 of whom were followed into the seventh month of their illness. The use of tartar emetic in such a large group of acute cases of schistosomiasis has not been previously reported.

MATERIAL AND METHODS

1. *Onset, diagnosis and incubation period:* All the patients in the present series had the onset of the acute symptoms in late January or early February of 1945. The diagnosis was made in all cases by the finding of mature ova of *Schistosoma japonicum* in the stool specimen. All patients were American soldiers not previously exposed to this disease. The average incubation period for 68 cases was 50.2 days.

2. *Severity of symptoms:* The incidence of acute symptoms and the physical findings parallel those reported by Billings, Winkenwerder and Hunninen. Five cases were severe, 31 moderately severe, 32 mild, and 4 were asymptomatic. The last group was discovered on routine stool examination.

3. *Treatment:* 1.89 gm. of tartar emetic was used in the treatment of this series. A solution of 0.4 gm. tartar emetic per 100 cc. of 5 per cent dextrose-physiological saline was made up under sterile conditions. The solution was never heated and was used within three hours of mixing. It was given intravenously every other day for eight-

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een doses. The first dose was 0.020 gm. of tartar emetic or 5 cc. of the solution. The second dose was 0.040 gm., the third dose 0.060 gm., the fourth 0.090 gm., and thereafter all doses were 0.120 gm. or 30 cc. of the solution. In the first four doses the solution was diluted with 5 per cent dextrose-physiological saline to make 30 cc. of liquid injected. It was given over a period of about ten minutes into the antecubital vein in a 30 cc. syringe.

3a. *Treatment reactions*: Chart number one shows that there were a great many minor reactions to treatment and four severe reactions necessitating discontinuance of the tartar emetic. The cough, nausea and vomiting were acute symptoms starting while the tartar emetic was being injected. The stiffness of the joints came on later and became worse gradually as more of the drug was given. The cough was prevented or decreased in many cases by giving 0.0006 gm. of atropine orally one half hour before injection.

3b. *Retreatment with 40 cc. of Fuadin*: Of the 72 patients who completed the course of tartar emetic, 32 were retreated with 40 cc. of Fuadin one month after the tartar emetic course was finished. These patients were retreated because degenerated eggs were found in their stools in the third and fourth week after tartar emetic treatment. None of these patients had immature eggs. Because the significance of degenerated eggs was not known, retreatment was thought to be indicated. The Fuadin was given in eight consecutive daily doses of 5 cc. by the intramuscular route.

RESULTS

All patients were discharged to duty after hospitalizations ranging from one to four months. Thirty-two were available for study throughout the first seven months of their illness. Of these 14 had been retreated with 40 cc. of Fuadin. Subjectively few of the discharged patients felt perfectly well, although all carried on light duty. Complaints such as headache, nervousness, lassitude, and weakness were common. Many of these symptoms were thought to be on a functional basis, arising in a situation of uncertainty about the future, associated with changes in disposition policy and long hospitalizations (3).

1. *Clinical observations during the first seven months*: There was no

striking improvement with the institution of tartar emetic therapy. Of 31 patients weighed in the seventh month of their illness, 35 per cent had lost weight since the onset of the illness.

CHART 1

Reactions to tartar emetic treatment (in 72 patients)

DOSE	COUGH	NAUSEA	VOMITING	STIFFNESS	MISCELLANEOUS
1	1	3	0	0	
2	1	3	1	0	
3	3	4	1	0	
4	11	7	2	1	
5	20	3	0	2	Purpura*
6	16	2	0	2	
7	13	5	2	2	Substernal pain*
8	15	7	3	4	
9	14	3	1	5	Vascular collapse*
10	16	4	2	5	
11	15	2	1	6	
12	17	6	4	7	
13	15	10	6	11	
14	16	4	0	19	
15	15	10	3	20	
16	16	9	3	28	
17	17	9	4	39	Cough, diarrhea, vomiting*
18	13	11	5	38	

* Severe reactions necessitating discontinuance of tartar emetic.

CHART 2

Change in physical findings with time

PHYSICAL FINDING	MONTH			
	1	2	3	7
General glandular enlargement.....	33*	35	6	—
Cervical glandular enlargement.....	31	19	13	—
Palpable liver.....	29	24	11	12
Palpable spleen.....	28	7	3	3
Total cases.....	72	70	62	31

* All figures except those in the lowest horizontal line are in percentages.

General glandular enlargement was not appreciable after the third month of the illness (chart number two). Cervical glandular enlarge-

ment persisted slightly longer than general glandular enlargement. Chart number two shows the hepatic and splenic indices in the first, second, third and seventh months.

2. *Laboratory observations during the first seven months:* Chart number three shows that the leucocyte count approached normal as time passed. Retreatment with Fuadin did not significantly influence the average leucocyte count as compared with the untreated group. The absolute eosinophile count also approached normal as time passed, as is shown in chart number four.

CHART 3
Fall in leucocytes with time

LEUCOCYTES	ONSET	MONTHS				
		1	2	3	4	7
<i>thousands</i>						
35+	3.2*					
30+†	3.2					
25+	3.2					
20+	12.9	3.2				
15+	24.3	12.7	1.5	5.5		
10+	37.1	42.8	19.7	10.9	19.7	12.9
5+	16.1	38.7	74.2	80.0	77.0	74.2
2+	0.0	1.6	4.6	3.6	3.3	12.9
Total cases.	62	63	66	55	61	31

All figures are in percentages except the column of the left and the lowest line.

* Two leucocyte counts were above 35.0 thousand. One was 54.9 and the other 45.9 thousand.

† 30+ represents all leucocyte levels between 30.0 thousand and the figure directly above it, in this case 35.0 thousand.

Twenty-six patients had hematocrits performed and 29 had sedimentation rates done in the seventh month, and all were normal.

Stool examinations were done twice weekly following treatment until the seventh month of the illness (fifth month post tartar emetic treatment). As time went on, patients were lost through rotation and troop movements. In the seventh month, all patients who could be reached were hospitalized and examined completely. There were 32 patients followed into the seventh month. Two patients showed mature eggs of *Schistosoma japonicum* in the sixteenth week post

CHART 4
Fall in eosinophiles with time

ABSOLUTE EOS. LEVEL	ONSET	MONTH				
		1	2	3	4	7
<i>thousands</i>						
16+	6.6*					
15+†	3.2	1.5				
14+	0.0	0.0				
13+	1.6	0.0				
12+	8.2	1.5				
11+	4.4	0.0				
10+	6.6	3.0				
9+	4.9	1.5				
8+	4.9	7.9				
7+	6.6	3.0				
6+	4.9	4.7	3.0		1.7	
5+	19.6	6.3	3.0		0.0	
4+	6.6	9.5	7.5		0.0	
3+	6.6	27.0	0.0		1.7	
2+	3.3	12.6	7.5		1.7	3.2
1+	3.3	9.5	37.9	27.3	25.4	9.7
0.2+	4.9	7.9	30.3	41.8	44.1	38.7
0.0+ (normal)	3.3	3.0	10.6	30.9	25.4	48.4
Total cases.....	61	63	66	55	59	31

All figures are in percentages except the left column and the lowest line.

* Four absolute eosinophile counts were above 16.0 thousand. They were 45.9, 25.8, 21.8 and 18.0 thousand.

† 15+ represents all eosinophile levels between 15.0 thousand and the figure directly above it, in this case 16.0 thousand.

CHART 5

Incidence of positive stools for mature ova of schistosoma japonicum in the months following treatment with tartar emetic (includes cases treated with Fuadin)

	MONTHS POST TARTAR EMETIC TREATMENT					
	0	1	2	3	4	5
Number of cases showing mature ova of <i>Schistosoma japonicum</i>	72	0	0	0	2	2
Total cases.....	72	71	71	42	25	32

tartar emetic treatment and 2 others in the seventeenth week (chart number five). Of these 4, 3 had been retreated with 40 cc. of Fuadin. Only one of this group had changes on sigmoidoscopy.

3. *Sigmoidoscopy*: All 32 patients available in the seventh month of the illness (fifth month post tartar emetic treatment) were sigmoidoscoped. Of these, 8 showed nodules in the rectum or sigmoid of the type described by Johnson and Berry (4). Only one of these had a positive stool. Four had been retreated with 40 cc. of Fuadin. All patients with positive stools or nodules in the lower intestine were considered to have recurrences and were retreated with 60 cc. of Fuadin in 5 cc. daily doses intramuscularly without any reactions.

DISCUSSION

An analysis of the cases having recurrences, as determined by either a stool or sigmoidoscopic examination in the seventh month, was made with regard to severity at onset, leucocytosis and eosinophilia at onset and in the seventh month, as well as the number retreated with 40 cc. of Fuadin. There was no significant difference between the cases which recurred and those which did not in regard to the original leucocytosis and eosinophilia or for these values at the end of seven months. Retreatment with Fuadin had no effect on preventing recurrences. None of the asymptomatic cases recurred. Otherwise, the clinical severity of the disease at onset did not influence the recurrence rate.

It was our impression that the patients treated with tartar emetic had fewer recurrences than a comparable group (1, 2) treated with Fuadin alone in the same hospital.

SUMMARY

Seventy-two cases of acute Schistosomiasis japonica were treated with intravenous tartar emetic. Thirty-two cases showing degenerated eggs in the third and fourth week after the tartar emetic course was finished were retreated with 40 cc. of Fuadin. Of the whole group, 32 were followed into the seventh month of their illness (five months post tartar emetic treatment). Findings on physical examination, on the leucocyte and eosinophile counts, as well as on stool examination, are present in chart form for the first seven months of the illness.

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THE CLINICAL USE OF PENICILLIN IN OIL AND BEESWAX IN PEDIATRIC PRACTICE

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Most of the reports concerned with the clinical application of penicillin suspended in peanut oil and beeswax (hereafter to be designated as P.O.B.) have dealt with its use in adults, particularly as a therapeutic weapon in gonorrhea and syphilis. Various authors (1, 2, 3) have clearly shown that uncomplicated gonococcal urethritis can be treated successfully with a single injection of P.O.B. Roman-sky (4), in a comprehensive review of 600 cases of various pyogenic infections treated with calcium penicillin in oil and beeswax, concluded that the results obtained were as satisfactory as with those treated by multiple injections of aqueous penicillin. There were forty-two cases of pneumococcal pneumonia included in his series, ten of which were in children under sixteen years of age. The results were apparently good inasmuch as only four of the patients required more than four days of treatment to clear the infection.

If one is able to maintain a satisfactory therapeutic level of penicillin for twenty to twenty-four hours by this method of injection, its clinical application in Pediatrics at once becomes apparent. As is well known, in children the associated symptoms of vomiting and failure to take fluids with almost all pyogenic infections sometimes make sulfonamide administration difficult. For this reason, many children have to be hospitalized for the sole purpose of aqueous penicillin therapy. The economic and therapeutic value of being able to treat these children at home with single daily injections of P.O.B. is quite obvious. With these objectives in mind a clinical study of P.O.B. was carried out in an active pediatric dispensary.

METHOD OF STUDY

Between January 1, 1947, and April 15, 1947, one hundred consecutive cases of pneumonia were treated with P.O.B. These patients

were completely studied with cultures, X-rays, and penicillin blood assays. In addition to the pneumonia group several other disease entities were treated with P.O.B., but without bacteriological studies. In these, only clinical impressions are available and will be discussed briefly later.

For the most part, these children were treated as ambulatory patients, necessitating daily visits to the clinic for their P.O.B. injections and follow-up care. Hospitalization was carried out only in the cases of severe pneumonia in which the treatment had to be supplemented with appropriate measures to correct associated dehydration, heart failure, cyanosis or severe anemia. Seven such children were hospitalized and received P.O.B. In addition, three of the patients on whom treatment with P.O.B. was unsuccessful were also hospitalized for sulfadiazine therapy.

The diagnosis of pneumonia was based on definite positive physical findings or X-ray evidence of pneumonic infiltration, or both. Once the diagnosis of pneumonia was made, nasopharyngeal and throat cultures were taken.

Throughout the study crystalline sodium penicillin suspended in peanut oil with 4.8 per cent (w/v) bleached beeswax was used. This was supplied in ten cubic centimeter vials containing 300,000 units per cubic centimeter¹. All patients received 5,000 units per pound at each injection.

The technique of withdrawing the P.O.B. from the vial and injecting the contents intramuscularly with the use of 20-gauge needles and dry sterilized Luer-Lok syringe was that advocated by Romansky (4). However, for the most part, the P.O.B. was not allowed to cool to room temperature prior to injection. We found that when giving relatively small doses to infants, the use of tuberculin syringes facilitated calibrating the amount given, with reasonable accuracy. All the injections were given intramuscularly, deep into the gluteal group of muscles. Subcutaneous administration was not used.

Treatment was carried out with the use of single daily injections until such time that the patient's clinical response was adequate and the temperature had returned to normal. Two further daily injections were then given and the child's course followed for one more week.

¹ The P.O.B. was kindly furnished by E. R. Squibb and Sons.

On the child's second visit to the dispensary, which was usually eighteen to twenty-four hours after the initial injection of P.O.B., blood was drawn for penicillin assay. Except for patients who were hospitalized, only a single penicillin assay was made on each child. With those children who were admitted to the hospital, two or three assays were obtained within a twenty-four hour period.

Most of the patients had chest X-rays taken on the first day of treatment. Thereafter, they were followed fluoroscopically and the X-rays were repeated in seven to ten days time on only those patients in which the response was either slow or a failure.

The final part of the study was carried out in an attempt to demonstrate any sensitivity that these patients may have developed to any of the constituent parts of the penicillin in oil and beeswax mixture. These results will be discussed in detail in a subsequent section.

THE PNEUMONIA SERIES

There were sixty-four males and thirty-six females in this group of 100 patients. Eighty-six were negroes and fourteen were white. Their ages ranged between two months and eleven years with fifty-seven of the children under two years of age, thirty-two of these being under twelve months. The remaining forty-three were in the age group between two and eleven years. Thus, a good proportion of the cases in our series were in the age group under two years, a group in which the mortality rate prior to the chemotherapeutic era ranged between twenty and thirty-five per cent.

Type of Pneumonia: For the purposes of clinical classification we have designated all cases with localized homogeneous consolidations as lobar pneumonia, while those with scattered patchy diffuse infiltrations were classified as bronchopneumonia. Under this rather broad classification the following types of pneumonia were observed:

	BRONCHOPNEUMONIA	LOBAR PNEUMONIA
Under two years of age.....	24	33
Over two years of age.....	11	32
Total.....	35	65

Etiological Agents: Hodes et al (5) have pointed out that in cases of pneumonia the pneumococci obtained from the nasopharynx were almost always of the same type as that recovered from the blood stream, empyema fluid; middle ear or cerebrospinal fluid of the same patient. They concluded that the pneumococci isolated from the nasopharynx were etiologically more significant than those recovered from the throat.

Results of our cultures for pathogenic organisms, the great majority of which were recovered from the nasopharynx, are tabulated below.

Pneumococcus type 4.....	4.	} Pneumococci present in 78% of the cases
Pneumococcus type 6.....	14.	
Pneumococcus type 14.....	19.	
Pneumococcus type 19.....	9.	
Pneumococcus type 23.....	5.	
All other types.....	27.	
beta hemolytic Streptococcus alone.....	5	
beta hemolytic Streptococcus with pneumococcus.....	14	
hemolytic Staphylococcus aureus.....	5	
<i>H. influenzae B</i> with pneumococcus.....	2	
<i>H. influenzae</i> (negative quelling types A and B) with pneumococcus....	7	
Negative cultures.....	6.	} 12%
Unsatisfactory cultures.....	6.	

Clinical Results: The clinical results of treatment were classified as either good, poor, or as failures.

A good result entailed an adequate response, with complete subsidence of the pneumonic infiltration along with clinical improvement in a reasonable length of time, with the only chemotherapeutic agent being P.O.B. Further subdivision of the good results into rapid and slow responses seemed of value. The response was considered rapid when the patient attained a normal temperature within forty-eight hours and was relatively asymptomatic within four days. All other patients who attained an ultimate good result, clearly influenced by the chemotherapy, but in a longer time, were considered to have a slow response.

Classified as poor results are those patients in whom there appeared to be an inadequate response to P.O.B. in the length of time that was required to bring the infection under control, or in which some complication, such as otitis media, developed while the P.O.B. was being given. Cases were classified as failures when sulfadiazine or aqueous

penicillin had to be given after the P.O.B. had failed to bring the pneumonia under control, or in which there was an exacerbation of the pneumonia.

As is indicated in Table I, 92% of the children had a good result with eighty-one responding in a rapid fashion and eleven slowly. There were four poor results and four patients that were classed as failures. There were no deaths in the entire group. In 96 cases, excluding the four failures, the temperature dropped to normal and remained there within an average of twenty-seven hours, with a range between twelve and one hundred and twenty hours.

This group of 100 patients received a total of 447 injections of P.O.B., averaging 4.5 injections per patient with a range of from two to nine injections.

TABLE I

RESULTS IN PNEUMONIA	GROUP
Good.....	92
Rapid.....	81
Slow.....	11
Poor.....	4
Failure.....	4
Total.....	100

Poor Responses to Treatment: Of the four patients for whom the results of treatment were considered to have been poor, all had rapid clearing of the original pneumonia. Nevertheless, these were considered to have shown a poor response because they developed otitis media under treatment. In two of these cases the organism was the same as that considered responsible for the pneumonia. In the third the otitis was due to *H. influenzae B*. All of these complications cleared slowly on sulfadiazine therapy. The fourth child had chronically infected adenoids with recurrent upper respiratory tract infections.

Failures: In four of the cases the treatment was considered as having failed for the following reasons. The first patient maintained fever and signs of consolidation after six days of treatment, but recovered rapidly under sulfadiazine therapy. His nasopharyngeal

cultures yielded type 15 pneumococci, *H. influenzae* and *Staphylococcus aureus*.

The second patient had pneumonia with severe dyspnea and cardiac failure. Although his temperature reached normal within twenty-four hours, signs of consolidation persisted at the left base throughout seven days of P.O.B. therapy. Cultures yielded type 14 pneumococcus and beta hemolytic *Streptococcus*. Nine days after therapy was discontinued, he developed fever and X-ray evidence of interlobar fluid. Following no response to aqueous penicillin he recovered under sulfadiazine therapy.

The third case was complicated by a nutritional anemia and chronic upper respiratory tract infection. Type 6 pneumococci and beta hemolytic *Streptococci* were recovered from nasopharyngeal culture. Because of only gradual improvement during six days of P.O.B. therapy, he was hospitalized and improved rapidly following blood transfusions and aqueous penicillin therapy.

The fourth patient was a mentally retarded two and a half year old child with pneumonia (*pneumococcus* type 14). His temperature fell to normal within thirty-six hours and he was symptom-free in three days except for continued nasal discharge. He was treated for four days but, three days after discontinuance of P.O.B., developed fever, increased nasal discharge and rales throughout the lungs. He had a good response to a second course of three daily injections of P.O.B.

In each of the above cases there was some complicating factor on which the apparent failure of treatment might well be explained. It is deemed wisest, however, to consider that the treatment failed to accomplish the desired result in these patients.

Complications: Non-suppurative catarrhal otitis media occurred as a complication in seven of the 100 cases of pneumonia. Six of these were present at the time of the first visit and the other appeared on the fifth day of treatment.

Suppurative otitis media was seen in seven of the patients. Four of these were present on admission, while the remaining three developed on the third, fourth and fourteenth days respectively. In general, it was found that when otitis media complicated the pneumonia, the treatment with P.O.B. had to be continued for two to three days longer than when this complication was absent. As was shown in three of the poor results, sulfadiazine had to be added to the therapy

in order to clear up the otitis media, although the pneumonia responded well to P.O.B.

Atelectasis of a single lobe of the lung occurred definitely in two patients and was questionably present in two others. Neither of the two definite atelectases, one in the right upper lobe and the other in the right middle lobe, required bronchoscopy. Both cleared spontaneously in five to eight days time. The two questionable atelectases, both of the right middle lobe, also cleared spontaneously.

TABLE II
Infections Treated with P.O.B.

DISEASE	RESULTS		
	Good	Poor	Failure
1. Suppurative otitis media	17	0	1
2. Suppurative otitis media Sul- fadiazine resistant	9	0	0
3. Severe Pharyngitis	10	1	0
4. Severe Pharyngitis and otitis media	7	2	0
5. Ulcerative Stomatitis	14	2	0
6. Furunculosis	2	1	0
7. Cellulitis	4	0	0
8. Suppurative Adenitis	4	0	0
9. Peritonsillitis	2	0	0
10. Conjunctivitis—Non-specific . .	2	0	0
11. Gonorrheal Vaginitis Sulfadi- azine Resistant	2	0	0
Total	73	6	1

The fairly definite interlobar empyema that came on while the patient was being treated with P.O.B. has already been discussed as one of the failures. There were no cases of meningitis complicating the pneumonias.

OTHER INFECTIONS TREATED

Several other types of infections were also treated with P.O.B., but inasmuch as the bacteriologic workup was incomplete, only clinical impressions are available. There was a total of eighty such infections treated, the results of which are tabulated in Table II.

The results, in general, were quite satisfactory, particularly in regard to the twenty-seven cases of suppurative otitis media, nine of which had failed to respond to sulfadiazine therapy. With only one exception the aural discharge stopped within two to three days after the institution of P.O.B. therapy and the tympanic membranes healed rapidly. Four to five days of P.O.B. injections were sufficient to bring about these results.

P.O.B. significantly reduced the duration of symptoms in ulcerative stomatitis. Within twenty-four to forty-eight hours after the first injection these children showed marked improvement. Three daily injections were usually all that was necessary.

PENICILLIN ASSAYS

The serum assay method used was that originally described by Rammelkamp (6), with modifications according to Eagle (7, 8).

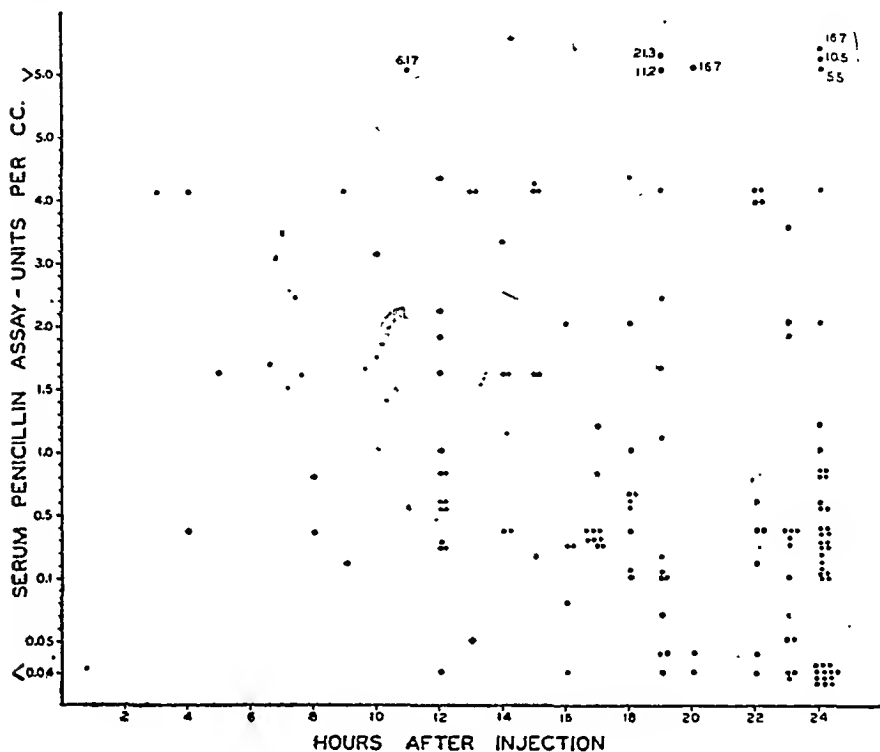
Graph I illustrates the composite picture of the 153 penicillin assays that were determined. One can see the tremendous variation in the levels that were present at any given hour. So great was the variability of these levels that we were unable to plot a mean curve which would be of any value. Of the seventy-one assays obtained in the period twenty-two to twenty-four hours after the injection of P.O.B., 18% showed no detectable titre (less than 0.03 units per cc.).

There are several possible factors that may be responsible for the great variability in absorption rate of penicillin when this agent is suspended in peanut oil and beeswax. First of all two different lots of P.O.B. were used throughout the study. In the latter half, the levels were much more consistent than during the time the first lot was used. The reasons for this discrepancy are not clear.

Secondly, Romansky (9) has shown that the rate of penicillin absorption is greatly dependent on the degree of muscular activity. If the P.O.B. is given in the late afternoon he has found that, with the decrease in muscular activity during the sleeping hours, there is a slower absorption of the penicillin during the first twelve hours. This then leaves a greater quantity of penicillin to be absorbed during the second twelve hour period, and consequently higher levels are obtained in the last four to six hours. Some of the variability in our patients' levels might well be accounted for along this line of reasoning,

for the injections were given at all hours during the day, the majority of them in the morning.

The factor of muscular activity and its consequent effect on the rate of absorption probably plays a more important role in children than in adults. It is difficult to keep a moderately ill child at low activity



GRAPH I

even in bed, although with seriously ill children activity is usually at a minimum. Therefore, it is reasonable to assume that there would be great variation in the absorption curves.

We must therefore draw the conclusion that it is impossible to be certain how long any given patient will have a titratable penicillin level following an injection of P.O.B.

REACTIONS TO P.O.B.

Romansky (4) in summarizing the use of P.O.B. on nearly 4,000 cases receiving 60,000 injections found only two instances of sterile

foreign body abscesses. Both of these were in patients who received the material subcutaneously. Allergic manifestations, characterized by reactions varying from slight urticaria to generalized angioneurotic edema occurred in about five per cent of his cases. It is Romansky's (9) opinion that these reactions with P.O.B. occurred only slightly more frequently than with the use of aqueous penicillin. There was no evidence of any paraffinomas developing in any of the patients in this large series of 4,000 cases.

Nichols and Haunz (10), in treating forty patients with P.O.B. given subcutaneously, found no allergic reactions and only two instances of mild inflammatory reaction at the site of injection. Such reactions are apparently much more commonly seen when the material is given subcutaneously rather than intramuscularly.

In our total series of patients, numbering 180 and representing 696 injections, we observed no generalized allergic reactions whatsoever. There were two instances of a mild inflammatory reaction with some induration of the surrounding tissue at the site of injection. One of these occurred when the material was given subcutaneously by mistake. Both of these reactions subsided spontaneously in two to three days time. As Romansky (4) has pointed out, this type of inflammatory change may represent a local allergic response.

Gay (11) has demonstrated the nonantigenic character of peanut oil-beeswax mixtures even in known pollen sensitive patients. However, with the slow prolonged absorption of penicillin afforded by suspending it in this peanut oil-beeswax medium, there is the possibility that these patients may develop sensitivity to the various components of the mixture, with subsequent allergic reactions.

With this possibility in mind, thirty-five of the children were tested for sensitivity two to four months after they had been treated with P.O.B. Each child received an intracutaneous injection of about 100 units of aqueous penicillin G and a similar injection of the peanut oil-beeswax vehicle. At the same time he was given an intramuscular injection of the same dose of P.O.B. previously used for daily treatment of his infection. These children were then detained in the clinic for one hour, during which time the skin tests were read and they were observed for any systemic reaction. At the end of one hour their temperatures were retaken and they were then sent home.

On return to the clinic the following day their temperatures were again recorded and they were examined for any sensitivity reaction. The visiting nurse services went into the homes twice during the three days of observation so that any of the allergic reactions would not be missed.

In this group of thirty-five children there was only one questionable allergic reaction. This was in a child of two years of age, who had a history of periodic attacks of urticaria. He had been treated for otitis media with P.O.B. two months prior to being tested. No allergic reactions were noted at that time. At the time that the skin tests were applied he had a few erythematous plaques scattered over the chest and abdomen. The skin tests were negative and he had no febrile response. Eight hours after being tested he was reported to have broken out with a wide spread urticarial eruption which lasted only about six hours. By the time he was seen the following morning the urticaria had completely disappeared. In view of the preceding allergic history, plus the fact that the skin tests were negative and the transitory character of the urticaria, we doubt that this represents a true allergic response to P.O.B.

The remaining thirty-four children elicited no allergic response, and all the intracutaneous tests were negative.

SUMMARY

1. One hundred and eighty children with various acute infections were treated, as out-patients, with penicillin suspended in a mixture of peanut oil and beeswax.

2. There were 100 consecutive cases of pneumonia, thirty-two of which occurred in infants under twelve months of age. The results achieved were quite satisfactory, with ninety-two good responses, four poor responses, and four failures.

3. Twenty-nine cases of suppurative otitis media, nine of which proved to be sulfadiazine resistant, were also treated. There was only one failure in this group.

4. The penicillin blood levels obtained by this method of injection were extremely variable. The possible reasons for this marked variability are discussed.

5. Two instances of mild local inflammatory reaction at the site of

injection were encountered. Both cleared spontaneously in three to four days time without abscess formation.

6. There were no allergic reactions to the injections in this entire series of 180 patients, who received a total of 696 injections.

7. No instances of acquired sensitivity to penicillin or the peanut oil-beeswax vehicle could be demonstrated by subsequent injection of these products two to four months later.

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SEDIMENTATION RATE IN ASTHMA

IN CHILDREN

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In a previous paper (1), the results of the treatment of a group of 34 asthmatic children by irradiation of the lymphoid tissue of the nasopharynx with radon were reported. The ages of these patients varied from 2 to 14 years. All of them had suffered with frequent asthmatic attacks for at least 2 years prior to irradiation. This group included patients with all types of asthma: intrinsic and extrinsic, as well as instances of the mixed or unclassified type. Approximately one-half of the patients had had their tonsils and adenoids removed some time before the institution of the radon therapy.

During the course of this study, erythrocyte sedimentation rate determinations (Wintrobe technique) were done on 33 of the 34 patients treated in this manner. Determinations were made before the application of the radon therapy, during the course of the treatment, and after the treatment was completed. An attempt was made to perform at least two determinations on each patient under the same conditions. Also, before the radon therapy was instituted determinations were done on each patient, first, when the patient was entirely free of asthma, and, second, when the patient was having an asthmatic attack.

The sedimentation rates of each patient after having been treated with radon are shown in the following tables in relation to the outcome of the asthma. Corrected values read in one hour are reported. In each table the figures in *italics* represent the sedimentation rates taken during an asthmatic attack; the other figures denote an average of at least two determinations performed under the designated conditions.

The period of observation of these 33 patients, after the completion of the radon treatment, varied from 6 months to 4 years. The effect of the treatment on the course of the asthma was scored as follows:

(1) Completely relieved, when there was no recurrence of the asth-

TABLE I
*Sedimentation Rates of Patients Who Were Completely
 Relieved of Their Asthma After Radon Therapy*

PATIENT NO.	SEDIMENTATION RATES (MM. PER HOUR)		
	Before treatment	During treatment	After treatment
1	18-22	20	8
2	26-24	7	8
3	36-38	11	12
4	42-34	16	5
5	26-36	32	7
6	46-39	23	0
7	26-32	18	7
8	21-28	15	4
9	22-23	9	2
10	26-22	9	4
11	27-36	12	6
12	11-19	15	0
13	28-29	16	6
14	42-36	20	8
15	38-22	14	7
Average.....	29-28	16	6

TABLE II
*Sedimentation Rates of Patients Who Were Markedly or Moderately
 Relieved of Their Asthma After Radon Therapy*

PATIENT NO.	SEDIMENTATION RATES (MM. PER HOUR)		
	Before treatment	During treatment	After treatment
16	9-17	12	7
17	11-20	14	10
18	17-21	11	9
19	11-24	25	16
20	38-41	6	5
21	29-41	13	0
22	11-7	4	11
Average.....	18-24	12	8

matic attacks during the observation period and repeated physical examinations showed none of the findings typical of the asthmatic chest.

(2) Markedly relieved, when the patient had only an occasional mild attack of asthma in comparison to the frequent severe attacks prior to treatment.

(3) Moderately relieved, when the number of attacks and the severity of the symptoms became less than one-half.

(4) Failure, when the number of asthmatic attacks was only slightly reduced or the status remained the same, or the condition became worse.

TABLE III
*Sedimentation Rates of Patients Whose Asthma Did
Not Respond Favorably to Radon Therapy*

PATIENT NO.	SEDIMENTATION RATES (MM. PER HOUR)		
	Before treatment	During treatment	After treatment
23	2- 4	5	8
24	3- 5	3	0
25	0- 1	0	0
26	0- 2	0	1
27	9- 1	9	0
28	8- 0	4	1
29	4- 5	9	0
30	5-12	16	10
31	4- 0	0	0
32	7- 2	3	1
33	10- 8	7	5
Average.....	5- 4	5	2

It will be noted that the sedimentation rate was essentially the same during both the asthmatic and the non-asthmatic states. Again, it will be observed that 21 of the 22 patients, whose asthma responded favorably to the radon therapy, had a high sedimentation rate before treatment. In all but one case these elevated sedimentation rates returned to normal after completion of the radon therapy. Of the 11 patients who failed to respond to the irradiation only one presented a slightly elevated rate before treatment.

COMMENT

These findings suggest, first, that the erythrocyte sedimentation rate determination may be a useful test in predicting which children

with asthma will be benefited by irradiation, and, second, that the sedimentation rate may serve to differentiate between infectious and non-infectious (allergic) asthma as has been pointed out previously by Coke (2) and Knott (3). This latter inference is further substantiated in this study by the fact that in all of the 22 cases whose asthma responded favorably to the treatment there was complete disappearance of the lymphoid tissue in the naso-pharynx, and again in all but one of these cases the sedimentation rate which was high before treatment returned to normal after the completion of the treatment.

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A HITHERTO UNRECOGNIZED TENDENCY TO THE DEVELOPMENT OF WIDESPREAD PULMONARY VASCULAR OBSTRUCTION IN PATIENTS WITH CONGENITAL PULMONARY STENOSIS (TETRALOGY OF FALLOT)

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The surgical treatment of congenital pulmonary stenosis recently devised by Blalock and Taussig (1) consists, as is now well known, in anastomosing a systemic artery (subclavian, innominate, carotid) to a pulmonary artery. By so increasing the amount of blood passing through the lungs, relief is afforded from the marked deficiency in the oxygenation of the blood that results from the pulmonary stenosis and from the shunting of unoxygenated blood from the right to the left ventricle through the ventricular septal defect that is usually present in these cases. The successful development of this operation has brought a large number of patients with the tetralogy of Fallot (congenital pulmonary stenosis, patent interventricular septum, dextro-position of the aorta, hypertrophy of the right ventricle) to this hospital for treatment. The condition of many of these patients at the time of their arrival at the hospital has been precarious. Some have died while awaiting operation. Others have been operated upon even though in a condition that made them extremely poor surgical risks, for the operation provided the only hope of their survival. Some deaths have represented the operative mortality to be expected in the development and performance of any operation of comparable seriousness, particularly in the presence of an abnormal cardiac mechanism. In the microscopical study of the tissues of two of the fatal cases there was encountered pulmonary vascular obstruction of a degree that could be of importance in contributing to the symptoms and, possibly, to the fatal outcome. It was regarded worthwhile, therefore, to examine a series of consecutive cases of the tetralogy of Fallot for the presence of these lesions, and they were found with a frequency that was altogether surprising (90 per cent of 21 cases).

The purpose of the present report is to call attention to this hitherto unrecognized tendency of these patients to develop widespread obstruction of the pulmonary vascular bed, for it is a matter that requires consideration in the interpretation of studies on the pathological physiology of the circulation in patients of this type.

The obstruction in question is the result of widespread focal thrombosis of pulmonary vessels of microscopic size. Both arteries and veins are involved, though in many instances it is impossible to decide whether the affected vessel is an artery or a vein, either because of the notorious similarity of the smaller pulmonary arterioles and venules (2), or because the wall of the affected vessel has been altered beyond recognition during the process of organization of the thrombus. Every stage of the process, from the formation of fresh thrombi to the organization and recanalization of older ones, is present (Figs. 1 to 10), and it is clear from the different ages of the thrombi in individual cases that the process is often a progressive one. Some of the older recanalized thrombi present an appearance suggesting minute cavernous angiomata (Figs. 4 and 5), but the abundant transition stages make it quite clear that lesions of this type are really the results of the recanalization of thrombi. This type of recanalization, leading to the formation of multiple, very wide channels separated by thin septa, is encountered only occasionally in the pulmonary vessels when thrombi undergo organization and recanalization in conditions other than pulmonary stenosis, and it is a very rare mode of recanalization of thrombi in systemic vessels. The mechanical conditions that favor its occurrence in cases of the tetralogy of Fallot are not clear.

It is especially to be emphasized that the thrombi in these cases tend to occur profusely throughout both lungs. In most of the cases in which they were present, they were found microscopically in the single routine section taken from each lung at autopsy. Further sections were made from each lobe of each lung in twelve of the cases, and the lesions were often found in the sections from each lobe. In some of the cases a remarkable number of vessels in the individual sections were involved. In one case (19506), as many as 91 cross sections of vessels containing organizing and organized thrombi were present in a single microscopic section measuring about 2×1 cm. The single section from each of the other lobes of the lungs in this case

contained, respectively, 35, 33, 19, and 14 obstructed vessels. The single section from each lobe of the lungs of case 19668 contained, respectively, 31, 18, 14, 10 and 4 obstructed vessels. The frequency with which the thrombi can be found in sections of only a few micra in thickness, taken at random from any part of any lobe of either lung, makes evident the profuseness of these obstructive lesions in most of the cases.

There arises the question why widespread thrombosis of the peripheral pulmonary vascular tree occurs with such frequency in patients of this type. It may be said at once that the thrombi are not simply results of the vascular operative procedure. While in some cases small thrombi may form at the operative site of anastomosis and become dislodged and swept as emboli into small branches of the pulmonary artery, it is to be emphasized that in most of these cases there is clear evidence that the thrombi occur independently of any operative procedure. In the first place, these widespread lesions are found in patients who have died while awaiting operation. In the second place, even in the operated cases thrombi are ordinarily present which are clearly much older than the survival time of the patient following operation. Completely organized, recanalized thrombi are found even in patients who died on the operating table, or within several hours after operation (see Table I). In cases 19561 and 19614, the only cases in this series in which the patients survived as long as $4\frac{1}{2}$ days after operation, there are present fibrous, recanalized thrombi that are without question much older than the postoperative survival time.

Since it is perfectly clear that patients with the tetralogy of Fallot tend to develop spontaneous thrombi which obstruct the smaller pulmonary vessels, one may inquire whether these thrombi arise locally in the pulmonary vessels or represent small emboli from extra-pulmonary sites. There is no evidence that the arterial thrombi represent emboli, and it is altogether unlikely that they do. No thrombi were found in the hearts; and in cases of other types in which thrombi in the heart or systemic veins serve as the source of pulmonary emboli, no such extremely diffuse obstruction of minute pulmonary vessels as is present in these cases is encountered. It seems evident from the study of the present cases that patients with the tetralogy of Fallot

TABLE I
Tetralogy of Fallot. 21 Consecutive Autopsies

AUTOPSY NUMBER	SEX AGE	THROMBI IN PULMONARY VESSELS			REMARKS
		Organized canalized	Organ- izing	Fresh	
19444	M 10 mos.	0	0	+	Died 1 day after operation
19448	M 4 yrs.	+	+	+	Died 12 hours after operation
19466†	F 5½ mos.	+	+	0	No operation
19470†	F 16 yrs.	0	0	0	No operation
19480	F 7 mos.	0	0	+	No operation
19486*	M 21 mos.	+	+	0	Died 1½ days after operation
19506	M 5 yrs.	+	+	0	Died during operation
19521	M 4½ yrs.	0	0	+	Died 1½ days after operation
19526	M 4 yrs.	+	+	0	Died 8 hours after operation
19561	M 11 yrs.	+	0	0	Died 4½ days after operation
19579	M 3 yrs.	+	+	0	Died 2 days after operation
19614	F 11 yrs.	+	+	0	Died 4½ days after operation
19661	F 7 mos.	0	0	+	No operation
19668	F 2 yrs.	+	+	0	Died 1½ days after operation
19691	F 8 yrs.	+	+	0	Died 1 hour after operation
19697	F 3½ yrs.	0	0	+	Died 12 hours after operation
19717	F 20 yrs.	+	0	0	Died 3 hours after operation
19770	M 2 yrs.	+	+	0	Died 2 days after operation
19799	M 8 yrs.	+	+	0	Died during operation
19821	F 2½ mos.	0	0	0	Died during operation
19835	F 8 yrs.	+	0	0	Died during operation

* Pulmonary stenosis; interventricular septal defect; transposition of aorta.

† Capillary angioma present in one section.

have a marked tendency to develop widespread local thrombosis of the smaller pulmonary vessels, both arteries and veins. It may be stated that the study of the other viscera in these cases disclosed no such tendency to diffuse thrombosis as was found in the lungs.

There are two circumstances that may be expected to favor spontaneous thrombosis in the pulmonary tree in these patients. In the first place, the anoxaemia due to the inadequate pulmonary circulation causes the development of a compensatory polycythaemia, often of marked degree, and polycythaemia is recognized as a condition favorable to thrombosis, apparently because of the increased viscosity of the blood (cf. the formation of "marantic" thrombi in patients with increased viscosity of the blood due to dehydration). Spontaneous thrombosis of systemic vessels (e.g., cerebral vessels) is familiar in polycythaemia vera. How frequently widespread thrombosis of the smaller pulmonary vessels occurs in polycythaemia vera cannot be stated with confidence at present, for the lack of mention of thrombi in the pulmonary vascular bed, in the description of most of the cases in the literature, does not necessarily provide assurance that they were not present. The little thrombi in their organized recanalized state can easily be overlooked, as is evident from the fact that they have heretofore been overlooked in cases of the tetralogy of Fallot. A re-examination of the several cases of polycythaemia vera available in the files of this department has disclosed no thrombi in the sections of the lungs, but the number of cases is too small to be of value.

In addition to the factor of polycythaemia, patients with the tetralogy of Fallot are subject to another influence, not present in patients with polycythaemia vera, that can favor the development of thrombosis in the pulmonary vessels, namely, the inadequate pulmonary blood flow that results from the pulmonary stenosis. It is well known that circumstances that tend to slow the flow of blood in a vessel predispose to thrombosis of the vessel; and the greatly reduced amount of blood delivered to the pulmonary circulation in cases of pulmonary stenosis, with the consequent low pressure in the pulmonary artery, may be expected to reduce the rate of flow in the pulmonary vessels. Because of this additional factor favoring thrombosis in the pulmonary vascular bed, a case of pulmonary stenosis having the same degree of polycythaemia as a case of polycythaemia vera should be more likely to develop thrombosis of the pulmonary vessels.

Since patients with the tetralogy of Fallot suffer from a marked deficiency in the oxygenation of the blood due to the reduced flow of blood through the lungs, the presence of widespread thrombosis of the pulmonary vessels may well add to the difficulty of oxygenation. The marked interference with the oxygenation of the blood that results from the obstruction of microscopic pulmonary vessels in cases of Ayerza's disease is familiar. The presence of obstructive thrombi throughout the lungs may also affect the outcome of the surgical attempt to increase the pulmonary blood flow by anastomosing a large systemic artery to the pulmonary artery. Whether the increased peripheral resistance in the pulmonary circulation, in cases with widespread obstructive thrombosis of the pulmonary vascular bed, will place an additional burden upon the abnormal heart following the surgical therapeutic anastomosis, can hardly be answered on the basis of present information, for the activity of the normal compensatory mechanisms is at present unpredictable in these patients with a congenitally abnormal circulatory apparatus. The answers to these and to other questions provoked by the presence of the obstructive thrombi must await studies of pulmonary pressure and flow in the living patient, correlated with the degree of thrombotic obstruction found at autopsy. The observations recorded in the present paper serve principally to draw attention to a frequent complication that should be borne in mind in the interpretation of studies on the pathological physiology of patients with the tetralogy of Fallot.

SUMMARY

Widespread and progressive thrombosis of pulmonary vessels of microscopic size is a frequent occurrence in patients with congenital pulmonary stenosis (tetralogy of Fallot type). This condition was present in 90 per cent of twenty-one consecutive cases studied at autopsy. It is suggested that the predisposing factors are the increased viscosity of the blood resulting from the compensatory polycythaemia, and the reduced rate of flow in the pulmonary vessels resulting from the greatly diminished amount of blood delivered to the pulmonary vascular system. The possible clinical significance of this widespread obstruction to the pulmonary circulation is mentioned.

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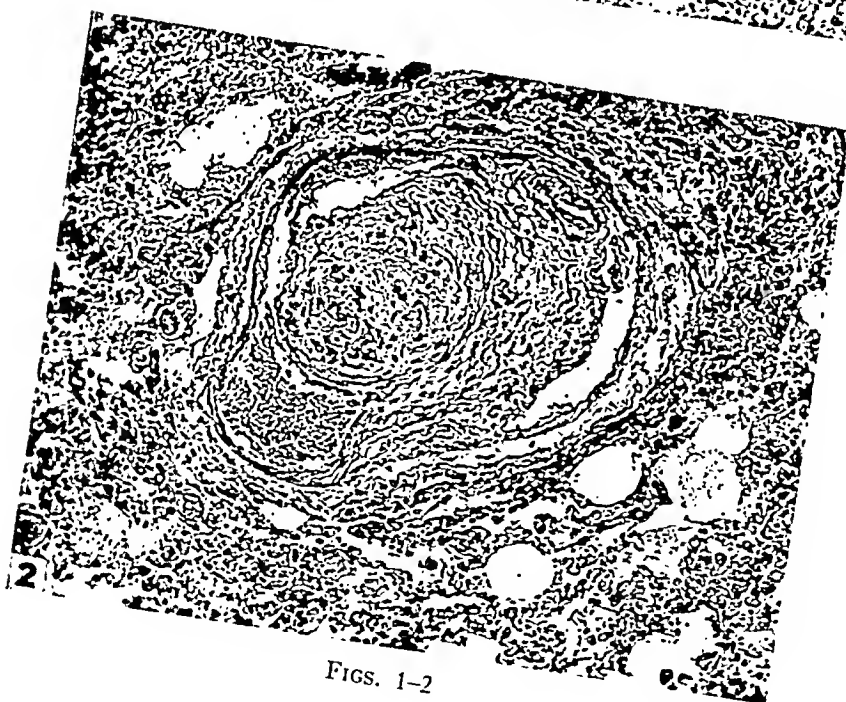
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EXPLANATION OF ILLUSTRATIONS

(Photomicrographs by Miss Marjorie Jackson)

FIGS. 1-5. Obstructed pulmonary vessels in Case 19668. Figs. 1 and 2 show thrombi still in the process of organization. Figs. 3, 4 and 5 illustrate older organized, recanalized thrombi.

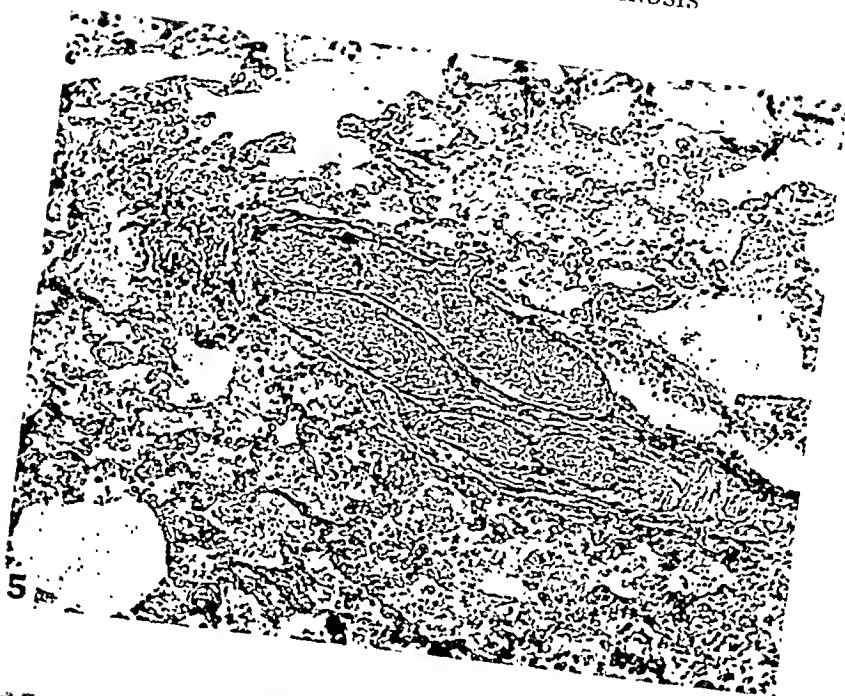
FIGS. 6-10. Obstructed pulmonary vessels in Case 19506. The lower left portion of the thrombus in Fig. 6 is not yet organized; those in Figs. 7-10 are completely replaced by connective tissue and recanalized.



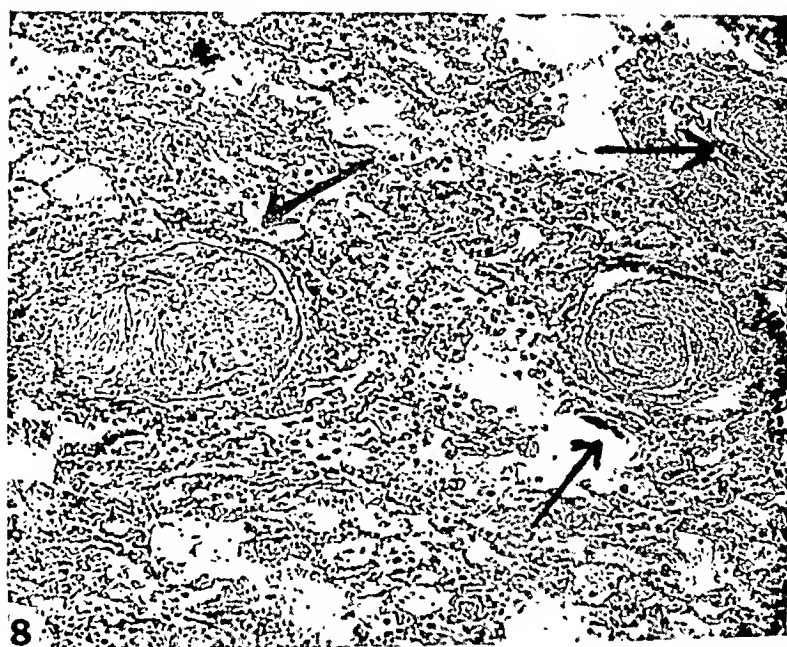
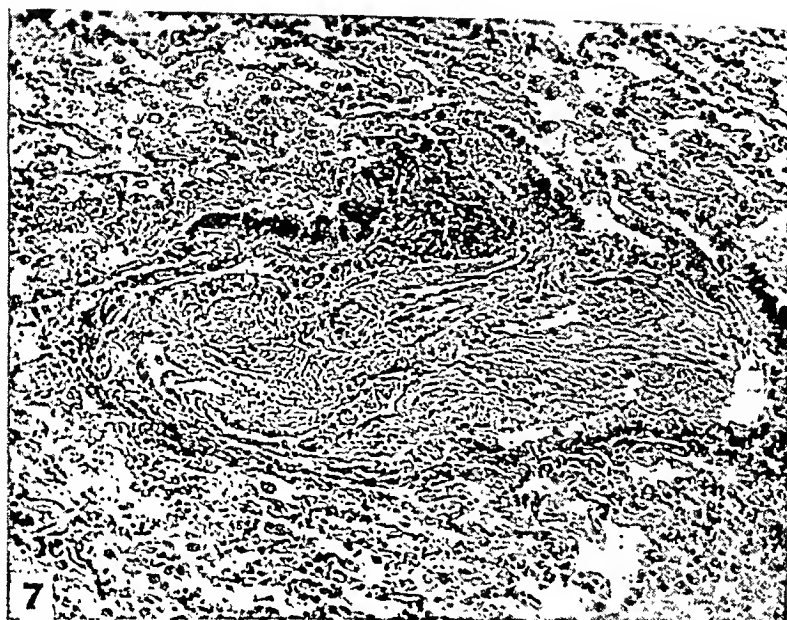
FIGS. 1-2



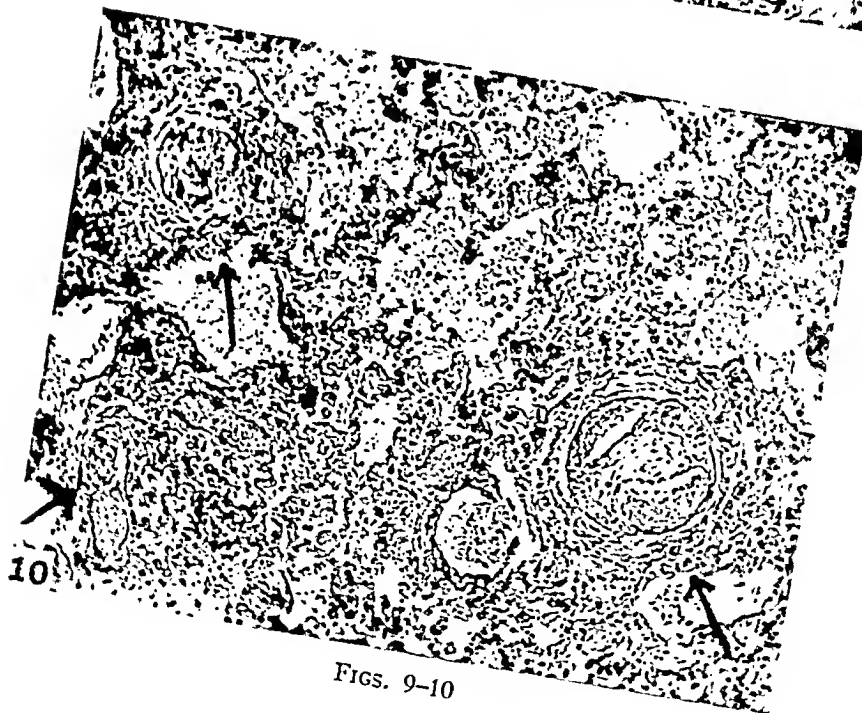
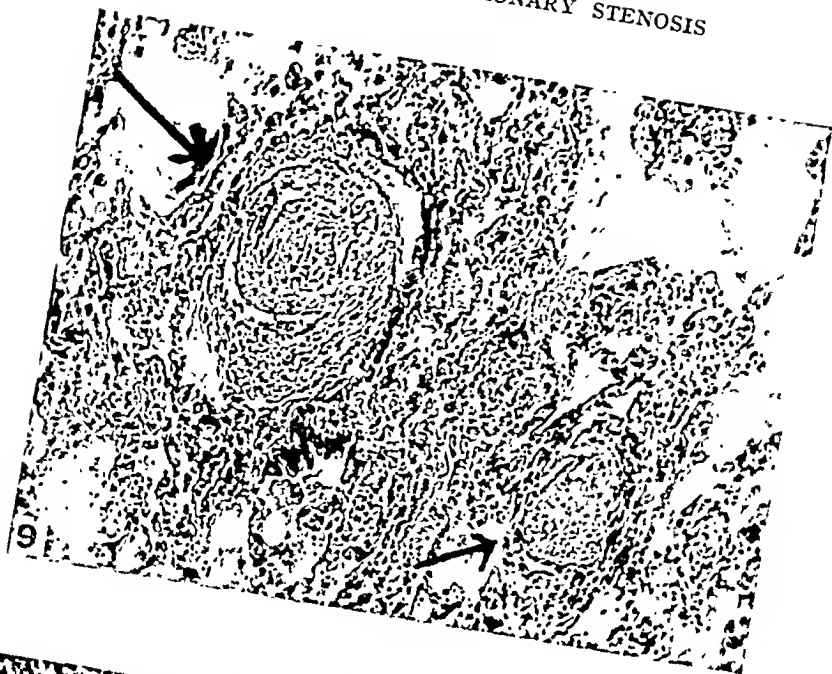
FIGS. 3-4



FIGS. 5-6



FIGS. 7-8



FIGS. 9-10

THE DOSAGE SCHEDULE OF PENICILLIN IN BACTERIAL INFECTIONS¹

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Although it is established with certainty that the administration of adequate amounts of penicillin by a dosage schedule which maintains a more or less constant concentration of the drug in the blood for a certain time will cure many severe and serious infections, it is by no means established that this dosage schedule is the most satisfactory or the only effective one. It is not easy to define the criteria that should be used to determine the most satisfactory or most effective dosage schedule of a chemotherapeutic agent. The first requirement should be the cure of all cases of infection or attainment as near as possible of that ideal. In addition, the number and severity of toxic reactions and the discomfort and inconvenience to the patient must be considered. The cost of and requirement of the doctor's and nurse's time in administering the treatment schedule might then be taken into consideration.

The dosage schedule of penicillin in general use has been arranged on the basis of three unproven assumptions; namely, 1) that it is necessary to maintain, day and night, a more or less constant blood concentration of drug for effective therapy, 2) that *in vitro* results on the sensitivity of parasites to the drug can be transferred without modification to infections in man, and 3) that it is a crime to use more penicillin than is necessary for the cure of the infection. We can consider the evidence for the validity or non-validity of these assumptions.

The concept of a maintained blood concentration of drug for effective treatment appears to have been a transfer by analogy from therapy with the sulfanilamide derivatives. Due to the extremely rapid excretion of penicillin by the kidneys, it was at first thought necessary to give a continuous intravenous or intramuscular injection of the drug

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to attain effective therapy. In fact, as recently as a month or so ago, we find the following statement in an article on penicillin: "The primary objective of penicillin therapy is the maintenance of therapeutic concentrations of the antibiotic in the body tissues" (1). One might have thought that the primary objective of any therapy was to cure the patient of his disease or to ameliorate disagreeable symptoms regardless of whether or not blood concentrations of drug are maintained.

Soon after the introduction of penicillin, the continuous intravenous or intramuscular drip was abandoned in favor of single injections at 2 to 4 hour intervals. This does not maintain a constant blood concentration. Due to the inconvenience of this schedule to both patient and attendants, various methods have been tried to prolong the blood concentrations that result from an injection of penicillin. These methods have attempted to decrease the rate either of its absorption or of its excretion. The most efficient method so far discovered of delaying absorption is the use of intramuscular penicillin in peanut oil-beeswax instead of in water. This slowing of absorption decreases the number of injections which have to be given in order to maintain a more or less constant blood concentration. The most satisfactory method of decreasing excretion is by the simultaneous administration of caronamide. This drug has not, however, been used primarily to decrease the interval between injections of aqueous penicillin, but has been used to decrease the amount of penicillin necessary for therapy. Its advantage is apparently a purely economic one of decreasing the cost of treatment. Where large amounts of penicillin are required in certain cases of infection, i.e., subacute bacterial endocarditis or where oral therapy is used, this may be an important factor. A marked disadvantage of caronamide is the very large daily dose of this drug which must be given.

As early as 1944, Tillett (2, 3) and coworkers reported results on the treatment of pneumococcic pneumonia which appear to show that the damaging effects of penicillin on invasive pneumococci restrained their regrowth for a considerable period after blood concentrations ceased to be measurable. They also state that when an interval of 12-16 hours was allowed to elapse between daily courses of injections the results were just as satisfactory as when the injections were maintained throughout the twenty-four hours. The number of cases studied

was small, but also the dosage of penicillin was small according to present standards. It may also be mentioned that in an article in 1944 on the "Clinical Use of Penicillin" Dawson and Hobby (4) state, "It should also be mentioned that little is as yet known concerning the minimal effective concentration for various types of infection. Such experimental work as has been done has been based on the assumption that effective concentrations should be constantly maintained in the circulating blood as is the custom in sulfonamide therapy. Certain facts suggest, however, that this may not always be necessary." In another article by McDermott et al (5) appearing in 1945, the following statement occurs, "There is also reason to believe from clinical experience and from certain of the *in vitro* experiments that this prolonged maintenance of an effective level need not be absolutely continuous."

We shall now discuss experiments on the treatment of bacterial infections in animals which may be of assistance in deciding dosage schedules of penicillin. It is of importance to note that a single dose of penicillin X will cure mice infected with a streptococcus (6), a phenomenon which cannot be duplicated with a sulfonamide. The experiments of Jawetz (7), published in January, 1946, are of significance. These appear to show that the effects of penicillin on the bacterial population in a streptococcus infection in the mouse last much longer than the measurable blood concentrations. The latter persisted for only about one hour after injection of the drug, while depression of bacterial population continued for eight hours or longer. It is to be noted that these experiments were done with a crude penicillin preparation of unknown composition.

Zubrod (8) in our laboratory has investigated the relative efficacy of different dosage schedules of penicillin in a β -hemolytic streptococcus infection of mice. Two samples of crystalline penicillin G, accurately documented and of high purity, were used. He found that, in treatment with multiple doses of aqueous penicillin, survival depends more on the total dose administered than on the number of fractions into which the total dose is divided. Although the blood concentration of the drug in mice is not measurable after about one hour, his experiments show clearly that nothing is gained by giving penicillin more often than once in twenty-four hours. The responses to a single dose cannot

be compared with those to multiple dose regimens because the dosage-response curves are not parallel—the single dose gives a much flatter type of curve, i.e., for each increment in dosage there is only a small gain in the number of mice surviving. These experiments by Zubrod give no evidence for the necessity of a maintained blood concentration of drug for effective therapy, but, on the contrary, suggest that the interval between doses of an aqueous solution of penicillin may be markedly increased and treatment still be highly effective in human bacterial infections.

A fact that must be considered in assessing the value of animal experiments on dosage schedules is that conclusions are based on calculated median curative doses, whereas we are more concerned with doses producing the maximum percentage of cures in human disease. The determination of doses producing effects in proportions close to one hundred per cent is difficult in that enormous numbers of animals are necessary to avoid a large error. In addition, one must recognize the hazard of transferring results obtained in the mouse to man without detailed knowledge of the pharmacology of the drug and the course of the experimental infection.

We can turn now to the second assumption upon which the dosage schedule of penicillin has been based. From a consideration of the mechanism of action of many chemotherapeutic agents, it seems unlikely that results obtained *in vitro* can be transferred to *in vivo* conditions with penicillin. However, one still finds this *in vitro* argument used for setting up a dosage schedule of penicillin (9). Some results reported by MacLeod (10) on pneumococcus infections in mice are evidence against the validity of transferring *in vitro* results to *in vivo* conditions. Infection was produced with strains of types I and III which had the same virulence for mice and the same sensitivity to penicillin *in vitro*. Infection with type I was much more easily cured in the mouse than was infection with type III.

The idea which is expressed in many publications, of defining the minimum dose of penicillin necessary for effective treatment and speaking of penicillin given in excess of this as so much waste, stems from the early days when the drug was so scarce that Florey recovered it from the urine of his patients. Also, lack of knowledge of the potential toxicity of the new drug led to conservatism in dosage. In order to

cure the maximum number of patients, dosage must be greatly in excess of the minimum curative dose for many individuals. Moreover, with an abundance of penicillin and with its purification to a point where such toxicity as is found is completely independent of dosage, waste becomes purely an economic matter. This economic factor is becoming of less and less importance.

In view of what has been stated above, it appears that there is a persistent antiparasitic effect of penicillin long after its disappearance from the blood. Hence, it appears probable that the maintenance of a more or less constant blood concentration is unnecessary for efficient therapy in the human. This means that it should be possible with aqueous penicillin given in three, two, or even one injection per day to accomplish satisfactory therapy. Probably very little increase in total daily dosage would be necessary. The advantage of such a dosage schedule over those where injections are given every two to four hours, or where injections of the peanut oil-beeswax preparation are used, appears obvious. Whether or not such dosage schedules can be used in all infections must be determined by careful, controlled clinical trials.

It may be mentioned in conclusion that in several clinics penicillin has been used successfully in aqueous solution with only two or three injections per day. This is in agreement with the argument presented above.

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PROCEEDINGS OF THE MEETING OF THE JOHNS
HOPKINS MEDICAL SOCIETY
HELD IN HURD HALL, NOVEMBER 10, 1947

Reduction of Intussusception by Barium Enema: Clinical and Experimental Study.
DR. MARK M. RAVITCH and ROBERT M. McCUNE, JR. (Department of
Surgery, Johns Hopkins Hospital).

The treatment of intussusception in infants and children by hydrostatic pressure has been routinely employed in reputable clinics in Europe and Australia for some 75 years. The results in terms of morbidity and mortality have from the first been superior to those reported from clinics using operation alone. In the Johns Hopkins Hospital since 1939 there have been 27 patients with intussusception who were treated primarily by barium enema. There were no deaths in this group and the average hospital stay was 9.2 days. During the same period of time there were 21 patients whose intussusceptions were treated primarily by operation with 5 deaths, or a 24% mortality. The living patients in this group had an average hospital stay of 32.4 days and a very much higher morbidity than the group treated by enema. It is fairly clear from the numerous reports of large numbers of cases treated by hydrostatic pressure that, with a pressure of 3 feet and without manipulation of the intestine through the abdominal wall, the bowel will not rupture and non-viable bowel will not be reduced.

An experimental method is described for the production of intussusceptions in dogs. Reduction of such intussusceptions by hydrostatic pressure of 3 feet confirms the clinical impression that non-viable bowel will not be reduced by a pressure of 3 feet. The histopathological features of intussusception are described. Bacteriological study showed several varieties of pathogenic organisms on the serosal surface of intussusceptions containing viable bowel.

Barium enema reduction of intussusceptions in infants and children by a surgeon, and in a hospital, is recommended as the primary treatment for the condition.

Dr. Alfred Blalock: Up to the time that Dr. Ravitch began this work, I had the same opinion in regard to the nonoperative treatment of intussusception as I had held about the nonoperative treatment of irreducible herniae. I remember so distinctly the views of a postgraduate student some years ago who stated that it was never necessary to operate upon irreducible herniae. In reply to a question as to how he treated this condition, he stated that he suspended his patients by their heels and gave them big dosages of apomorphine. According to this student, this resulted in vomiting and in a reduction of the hernia. It is obvious that this student was unwise in his choice of therapy, and I rather felt this way about the nonoperative treatment of intussusception until Dr. Ravitch convinced me of the safety of the procedure in his hands. I think that anyone using the method which he has described should realize that it is not devoid of danger in patients

who have an impairment of the blood supply of the intussuscepted part. Certainly the results which Dr. Ravitch has obtained are excellent, and I wish to congratulate him on this study and on the manner in which he has presented it.

Dr. Mark M. Ravitch: I think we would be perfectly satisfied if all those who are uncertain of their ability to diagnose the completeness of reduction would treat every case with enema and then routinely make a McBurney incision to confirm the completeness of the reduction and to see the condition of the bowel. I think the mistaken diagnosis of complete reduction would certainly be the greatest danger. Unless the bowel is manipulated or unless excessive pressure is used there appears to be very little danger of rupture.

Studies on the Diuretic Action of Diacetylformoguanamine in Dogs. DR. ELLIOT V. NEWMAN, DR. JOHN FRANKLIN, and DR. JACQUES GENEST. (Department of Medicine, Johns Hopkins Hospital).

From a large series of chemically related compounds Lipshitz selected melamine, adenine, and formoguanamine as having high diuretic activity by a test on rats. The purpose of our study was to determine the mechanism of action of diacetylformoguanamine (DAFG) in dogs. Adult female dogs weighing 13 to 18 kilograms were kept on a constant weighed diet of known composition. Total 24 hour collections were made of urine which was analyzed for sodium, chloride and potassium content. The dogs were weighed daily.

The results showed that when 0.5 gram of DAFG was given orally for one day with water provided ad lib, there was a rise in urine output of from three to five fold the control value. Intake of water usually kept pace with the output. There was little or no increase in the total 24 hour amount of sodium, chloride, or potassium in the urine and no weight loss.

When water was restricted to a normal volume (determined from the intake during the control periods), or when water intake occasionally did not quite keep pace with the output, there was a marked diuresis of from two to four times the control output of water and there was a marked increase in sodium and chloride output, and a moderate increase in potassium output. The dogs lost as much as one kilogram in weight in one day.

Administration of one to one and a half grams of DAFG for one day frequently caused a diuresis lasting two to four days, diminishing each day after the large initial diuresis. A post diuretic retention of sodium, chloride and potassium was marked after the first day of diuresis whenever water was restricted.

Administration of pitressin in large doses with DAFG did not alter the magnitude of the diuresis obtained. Crystalluria was noted occasionally in the catheterized urine specimen the morning after DAFG administration. The crystals were brown, oval or round, with coarse concentric rings and fine lines radiating from the center to the periphery. No hematuria nor albuminuria were noted. These crystals were found

only after careful routine search in morning catheter specimens which were centrifuged.

Administration orally of one gram a day of DAFG for five days caused, after the third day, a progressive drop in the creatinine clearance, para-amino hippuric acid clearance and T_m to twenty per cent of control normal values.

There was a rise in urea nitrogen. No suppression of urine output occurred at any time, a diuresis being sustained throughout the period of administration. Two weeks after discontinuing DAFG administration, all renal function tests mentioned above had returned to normal.

Another dog was sacrificed after five days of DAFG administration, when there was evidence of suppression of renal function tests but no suppression of urine output. Histological examination of the kidneys revealed a few of the crystals described above, with areas of dilated tubules and areas of normal kidney. The crystals were impacted in the tubular lumen.

Dr. Samuel P. Asper, Jr.: Dr. Newman, using diacetylformoguanamine, has confirmed the studies of Dr. Lindley on the diuretic action of these related compounds, and has given us a very good idea as to the mechanism of their action. In Boston, Dr. Myers, Dr. Williams, and I were interested in formoguanamine, and we made a few clinical observations on the use of this compound which is similar to the compound Dr. Newman just described. In six normal subjects and in nine patients with edema we were unable to obtain any significant diuresis. At times it seemed a slight diuresis occurred, but the results were neither consistent nor reproducible, and, furthermore, were of no therapeutic significance. In oral doses of 0.2 to 3.0 grams, we always obtained crystalluria with formoguanamine. These crystals vary in size, are visible in the urine to the naked eye, microscopically are green-brown, have radial striations and concentric markings. Under polarized light they are doubly refractile. In one patient, 1.6 grams of formoguanamine was administered on two successive days. There was no significant rise in the urine output over a control volume. There was no change in weight. On another occasion, 3.0 grams of formoguanamine was administered. Crystalluria became marked and the urine output fell to 200 cc. for two days thereafter. The urine sediment showed numerous red cells, white cells, occasional hyaline casts, and some of these casts had crystals attached to their surfaces. We were disappointed with the clinical experience with formoguanamine, and thought perhaps that there might be some different metabolic fate of formoguanamine in human beings as compared to that in animals—rats and dogs—that Dr. Lindley used.

Dr. Arnold R. Rich: I don't know whether Dr. Newman meant to imply that I concurred in the opinion that the kidneys of the treated dogs were normal. Certainly I would say that the picture of the first one looked very decidedly abnormal to me. The tubules were tremendously dilated and some were plugged with crystals. In the second lantern slide, one of the tubules seemed to be full of pus cells.

Dr. Elliot V. Newman: I should say that there have been other clinical studies which are rather conflicting and difficult to understand. There is at least one

report published, and maybe others, which say that formoguanamine is an effective diuretic, and I understand it is being widely used in some places. This is perhaps quite dangerous, as Dr. Asper has pointed out, and unless one looks carefully for crystalluria and other manifestations of renal damage one may not find it. Regarding the histological sections, I should have said that the area shown in the lantern slide, with the crystals in the tubular lumen, was abnormal as Dr. Rich has pointed out, but that numerous areas in other parts of the kidney were perfectly normal. The main pathological lesion seems to be one of focal obstruction due to crystals impacted in the tubules.

The Roentgenologic Diagnosis of Tuberculosis. DR. RUSSELL H. MORGAN. (Department of Surgery, Johns Hopkins Hospital.)

Since the introduction of mass radiographic methods for the early detection of tuberculosis, considerable concern has been raised as to the efficiency with which these methods discover tuberculosis. Within recent years, two comprehensive investigations have been carried out to determine this efficiency. One method employed a system wherein comparative surveys of the various methods were studied. The other method followed quantitative evaluation of the physical factors controlling the diagnostic quality of x-ray films of various sizes. Both these methods have proven conclusively that all the mass radiographic methods are as efficient as regular 14 x 17" films in the early detection of tuberculosis. It therefore can be confidently stated that the concern which has been felt regarding the usefulness of mass radiographic methods is unfounded and that these methods can be used without question in studying such groups as hospital employees and university student and faculty members.

Dr. Moses S. Shiling: I have been reading small chest films now for several years, and I would like Dr. Morgan to comment on the speed at which these small films can be read. I often hesitate to admit how fast one can read them. Not infrequently, 1000 to 1200 small films can be read in one hour without sacrificing accuracy.

It is difficult to understand how one expert can read a film as far advanced and another read the same film as negative. I can visualize several situations where this difficulty might arise in interpreting small films. One is the case of the large shelled-out cavity involving only a rim of tissue adjacent to the pleura. In this condition one might miss the complete absence of lung markings. Another similar situation could arise in the interpretation of cavities occurring in the so-called hilar regions of the lungs. These may well be picked up by some and missed by others.

Dr. A. McGehee Harvey: I would like to ask what the variations were when the same reader read the films on successive days.

Dr. Russell H. Morgan: In regard to Dr. Shiling's question about the speed of reading, some experimental work on evaluating diagnostic efficiency has been done as part of the Veterans Administration study, and it was apparent that the subjective error of reading is not very closely related to the speed of reading. Those

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who read at rates of upwards of 800, 900, and 1000 films an hour did apparently just as well as those who read at much slower rates, such as 100 or 150 films an hour.

Now as far as the question of missing far advanced tuberculosis is concerned, sometimes I think that that can be explained upon differences of designation. Frequently a process may be far advanced from the standpoint of its extent but looks so fibrous that the reader, without any bacteriological knowledge, diagnoses it as negative for tuberculosis, whereas the more careful reader a few minutes later will see the same picture and wisely, since he knows nothing of the bacteriological process involved because the film has no history attached to it, diagnoses it as a far advanced case. I think there is a good deal of that sort of thing in this reading. It is a subjective type of error, regardless of what you want to call it.

Now as far as readers who read the films twice are concerned, strangely enough there was as much discrepancy between two readings by the same reader as that which occurred with a number of readers reading the films once. In other words, if Dr. Smith (there wasn't any Dr. Smith in the study) read the series of cases one day, and then reread them the next week, his discrepancy from one week to the next was in the order of about 15%, the same as the discrepancy between five different readers reading films simultaneously. This subjective error is apparently a failing of human beings. I can't be too concerned with it. I am sure that if the records in the past year were inspected in our department and checked with final pathological interpretations on those cases, many films which I have read as negative would actually reveal pathological changes in many instances. There are a great number of causes for them of course—fatigue of reading, the pressure of other activities, an inadequate amount of clinical information. All of these may throw us off the track or not lend sufficient emphasis to certain things that would have noted if we had been tipped off ahead of time. Those are the things that are going to cause error. We do our best to hit the mark as often as possible; at least we keep our eyes as close to it as we can. Furthermore, I suspect that if it were possible to analyze carefully the clinical cases that come on the floor of this hospital, the best of our clinical personnel would make discrepancies in a certain number of cases. I am sure it is not a failing of radiologists and chest specialists alone.

Dr. Arnold R. Rick: I am sure that we all have great admiration for Dr. Morgan's simple and sensible method of evaluating the different types of film. While it is clearly a more objective method than has heretofore been used, I would like to ask Dr. Morgan how much subjectivity enters into it. At a given distance, for example, did it happen that one man would say, "I can't see any lesion", and then, after hearing the description of the lesion by the other two, would become able to distinguish it himself? Also, was there any attempt made by three men together to evaluate a group of films by the same method, without knowing in advance whether lesions were present or not? I am just interested in knowing how much of the process was subjective.

Dr. Morgan: I am sure that there is some subjectivity that comes into the picture. However, since we knew that the lesion was actually there, psychological factors involving competition among the various readers did not immediately come into play. I will say one or two words in regard to your first question—"What happens when the opinions vary; did the situation occur when two readers saw the lesion and a third did not?" That occurred not infrequently, as you would expect statistically, and whenever it did occur we abided by the majority rule. One interesting thing that came up in that connection was this: we observed, let us say, a group of films of moderately advanced cases at 10 meters. If a case came up in which one of us could not see the lesion and the other two could, we found that if the non-seeing reader went a half a meter closer to the film he could then usually pick up the lesion. Reversely, if the two went a half a meter farther back they might fail to see the lesion. The threshold between seeing and not seeing was fairly critical. In other words, in spite of the suggestion that this is not an entirely objective method, I think that it is fairly close to being one. One thing that I might point out, if there are any statisticians present, I think that we have been successful in showing that, in by far the largest percentage of instances, all of the methods not only are able to pick up the lesions that we studied but are likely to pick up lesions much finer than those we studied. From the charts you noticed that the ability of our 14 x 17 film to record detail was a great deal better than that necessary to pick out the finest of the processes. The shoulder of the curve was a long way away from the resolving power of the film. I think, as far as the diagnosis of tuberculosis is concerned, x-ray procedures are on pretty sound ground so long as the interpreters exercise the best possible diligence in observing the films. One other important thing is that the observers should not try to evaluate the activity of the lesion as soon as they detect the pathology. As soon as the readers attempted to do this in the Veterans Administration study, the discrepancy varied markedly. It is not surprising that this situation exists because x-ray methods are not histological methods, nor bacteriological methods, and the evaluation of the accurate status of the lesion must be determined by those methods rather than by x-ray. X-rays have done an admirable job in detecting the pathology. I think we should be appreciative of that fact. There are other excellent methods by which activity may be evaluated.

PROCEEDINGS OF THE MEETING OF THE JOHNS
HOPKINS MEDICAL SOCIETY

HELD IN HURD HALL, DECEMBER 8, 1947

Diagnostic and Therapeutic Technique Used in Hearing Rehabilitation. DR.
WILLIAM G. HARDY. (Department of Otolaryngology, Johns Hopkins
Hospital.)

The incidence of hearing disability is analyzed, and emphasis is placed upon the fact that only a small proportion of the affected group has as yet received adequate treatment. The recent growth of the field of audiology is discussed, and pertinent data, relating to clinical developments and psycho-acoustic research, are outlined.

The problems and methods of the field are given concrete demonstration by an analysis of the Navy's program of aural rehabilitation established in World War II. In connection with this program, lantern slides of physical facilities and certain research summaries are presented. The point is made that the clinical experience gained during wartime in the processing by the Army and the Navy of 16,000 patients with a handicapping hearing loss, offers rich resources for the development of similar clinical services for approximately 8,000,000 civilians with similar problems. (Of this patient-load of 16,000, the Naval center has handled 5,000 active-service personnel and veterans.)

On the basis of this wartime experience, certain pertinent generalizations are adduced:

1. Hearing rehabilitation is largely concerned with the treatment of a communicative disorder caused by hearing disability.
2. This disorder is psycho-social in nature and scope.
3. Contrary to general belief, the purchase of a hearing aid is by no means a sufficient answer to the needs of the hard-of-hearing person.
4. Adequate hearing rehabilitation must be organized in terms of three basic steps:
 - a. Thorough clinical diagnosis, employing objective measurements of auditory acuity and emphasizing the relationship of the communicative disorder to the behavior of the individual.
 - b. Careful selection of the hearing aid most appropriate to the individual's needs by means of adequate objective and subjective measurements and evaluations.
 - c. A retraining course in communicative skills, including auditory retraining, speech reading, speech training and mental hygiene,—the entire course gauged in terms of each individual's specific needs.

Certain special problems relating to psychogenic involvements and the development of language in children are outlined, and the relationship of hearing rehabilitation to the conservation of hearing is mentioned.

Pertinent research needs involving otology, psychology, physics, electronics and psycho-acoustics are suggested.

Dr. Stacy R. Guild: Besides congratulating Dr. Hardy on his report of the work in which he participated during the war, I wish to point out one aspect of the application of rehabilitation techniques to civilians which Dr. Hardy did not state. The broad application of this program will reduce the cost to the taxpayers of educating hard-of-hearing children. The cost of preventive measures for deafness and of fitting children with hearing aids will not be nearly so great as the cost of special educational procedures, special schools for the deaf, special teachers for the deaf, etc. I believe that Dr. Hardy's cooperation with the Department of Pediatrics and with city and state-wide agencies will more than make up its cost. We can call it preventive medicine if we wish.

Dr. William G. Hardy: I can supply one figure with which I am acquainted. Among several states on the Atlantic seaboard at least \$1300. to \$1800. per individual is spent annually among congenitally deafened children. I don't know what percentage of these children need not be in that group but it is probably a significant percentage.

Dr. Alan M. Chesney: I would like to speak for a minute from the standpoint of the guinea pig. I was very much interested, naturally, in Dr. Hardy's paper, and especially in his work which is starting here. I think it appropriate to tell this audience that the opportunity to start this work was made possible through the generosity of several of Dr. Crowe's patients, one of whom is himself obliged to wear a hearing aid.

It is quite true that the hearing aid of today is much better than it used to be. The old carbon-filament type of instrument was very unsatisfactory, but with the introduction of the radio amplification tube the whole picture changed. The only difficulty now is that so many improvements are being made and they are so expensive that you can't get a new model each year without spending a great deal of money. Maybe that will be straightened out as time goes on.

There is one point I would like to bring up that should be a matter of interest to all of us who are in the position of being medical teachers or medical students, and that is the question of the development of the amplifying stethoscope. I have watched its development with some interest, and I listened last year with the aid of an amplifying stethoscope to chests and hearts for a brief period and was amazed at what I could hear. I am satisfied that I heard things I didn't hear when I was a student, and I wouldn't be at all surprised if the development of amplifying stethoscopes reaches the point where all physicians starting out will want to have such a stethoscope and not wish to make use of the relatively inferior instrument which medical people have been using for a long time. If I were a youngster going into medicine now, I would keep my eye on that aspect of physical diagnosis. I rather think that a physician, with an amplifying stethoscope, may be able to hear things which his colleagues can't hear and which are really there and not imaginary.

It is very nice to have this work starting here under Dr. Hardy's supervision.

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Dr. John C. Whitehorn: I would like to make a very brief comment on one angle of this work as related to the field of psychiatry. In recent years we have stressed very much the importance of taking into account factors of human nature in the adjustment many people have to make to medical and surgical conditions. I am looking forward with much excitement and pleasure to participating and sharing in the results of Dr. Hardy's work here, as he has indicated tonight that his work has necessitated a very real taking into account of people's attitudes and the emotional stress which may hinder their best use of the apparatus available. I think we in psychiatry have an opportunity to learn quite a lot about normal human nature through the chance that Dr. Hardy will give us to have a look-in on his enterprise.

A Physiological Basis for Nervous Dysfunction. DR. W. HORSLEY GANTT. (Department of Psychiatry, Johns Hopkins Hospital.)

The organism tends to preserve itself and to adapt both individually and as a race, a function which has been well elaborated in Darwin's evolution and Cannon's homeostasis. This tendency of the organism to maintain an equilibrium through the higher nervous activity has been clearly demonstrated by Pavlov in his concept of the conditional reflex—a mechanism expressive of the individual's ability to adapt to the changes in its external environment.

A comparative study in my laboratory of the cardiac conditional reflex—the existence of which has been established here—with the salivary and the motor conditional reflexes ("crs") brings to light another and opposite basic characteristic of the organism, viz. the tendency to maladaptation or nervous dysfunction. The cardiac cr is *par excellence* a measure of emotional function. A comparison between the cardiac crs, with the secretory and with the somatic function of skeletal muscular movements, reveal two elements of dysfunction: 1) excessive cardiac reactions acquired through the experience of the individual, out of proportion to the biological needs, and 2) a persistence of these acquired cardiac crs after the more superficial crs have disappeared because of a change in the situation—a persistence which represents a failure of adaptation in the emotional nature, although there has been an adaptation in the more external aspects. We thus have to admit a basic function inherent in the organism making for maladaptation. A recognition of this dysfunction explains much of our normal nervous imbalance as well as the more extreme examples—neuroses and psychoses.

Dr. John C. Whitehorn: Those of us who have the responsibility of working clinically with people whose emotional functions are disturbed learn through difficult and complicated clinical experience the importance of those persisting maladaptive emotional tendencies which keep on acting in a person even when he knows better, so to speak—a situation strikingly analogous to that of dogs, which Dr. Gantt has spoken of, whose hearts have a conditional reaction different from that of their salivary glands and motor apparatus. We develop a way of thinking and talking about these matters in terms of emotional conditioning which is inter-

nalized and persistent. It may well be that through the type of experimentation which Dr. Gantt pursues diligently, we may be granted a more intimate view of the physiology underlying these persistent maladaptive emotional or cardiac patterns. This would be of very great importance for the understanding of persisting anxiety reactions. I can't stop without yielding to the temptation to speak of a further complication clinically which exists on top of this matter, namely that we do not infrequently deal with people who have found a solution, apparently not available to some of these dogs, whereby they become immune in a pathological way to the upsetting implications of some of the situations, but do this by delusion formation or by other devices which aren't very well adaptive either, so that the clinician oftentimes considers it a triumph therapeutically to get the patient to a state where his heart will respond. Sometimes he will have some moderately appropriate reactions and then there is a possibility that he can be helped still further.

Dr. Harry A. Teitelbaum: Since the process of extinction as described by Dr. Gantt apparently relieves animals, including man, of the necessity of responding to stimuli which are no longer of biological value, it must play a very important role in adaptive mechanisms. This presents the possibility of an interesting thesis. Whether Dr. Gantt's observations corroborate this thesis or not, it is impossible to say at this time. About all we can say about it this evening is that it seems that the more highly specialized tissues like the salivary glands and striated muscle tend to extinguish conditioned responses more readily than the less specialized tissues like the heart and the lungs which are more essential for the vitality of the animal. Can one postulate that the more specialized tissues are more highly sensitive and become conditioned more readily, and therefore play a more important role in adapting an animal, than the less specialized tissues in which the conditional responses are not extinguished and persist perversely and clinically in man? Referring to anxiety or emotional disturbances, it is possible that these less specialized tissues are less likely to extinguish their conditioned responses, and therefore are less adaptive. I wonder if Dr. Gantt could expound on these thoughts and tell us whether it is a tenable thesis or not.

Dr. W. Horsley Gantt: I am glad to have Dr. Whitehorn's and Dr. Teitelbaum's comments and the amplifications. I don't know that I can say anything especially that would illuminate the matter further than they have already done. I just want to add that necessarily I have condensed and, to a considerable extent, oversimplified this in order to give you a clearer idea than would have been done if I had put in a great deal of more complex material, and I offer it as a hypothesis which future work may confirm or reject.

Chemotherapy of Tuberculosis in Children. DR. EDITH M. LINCOLN. (Department of Pediatrics, New York University.)

A brief report of results with promizole includes the failure to cure five children with tuberculous meningitis with this drug, and our comparatively good results in

acute generalized miliary tuberculosis. Seven cases of the latter were treated for more than a month, and five of these are now free from x-ray evidence of miliary tuberculosis one to three years after the original diagnosis.

Streptomycin has been used in various forms of extrapulmonary tuberculosis with results similar to those obtained by other investigators. It has proven particularly valuable in curing pharyngeal and endobronchial tuberculosis and in promoting closure of draining sinuses. Streptomycin has produced rapid improvement in pulmonary tuberculosis of the so-called reinfection type but no cures. Patients with tuberculous meningitis were not treated with streptomycin alone, but seven cases were treated with streptomycin together with promizole. Six of these have survived for four to eight months, are normal mentally, and have no evidence of permanent neurologic damage. These cases have not been under observation for a sufficient length of time to be reported as cures. This is merely a preliminary report on combined therapy of tuberculous meningitis with streptomycin and promizole.

Dr. E. K. Marshall: The first attempts to use chemotherapy for tuberculosis were started in 1899 by Cornet, who treated guinea pigs with mercuric chloride. I think one only has to listen to what Dr. Lincoln has told us to realize what enormous advances have taken place in the last decade, in spite of experiments with thousands of compounds of all types which have been done in the past on experimental and human tuberculosis. There seems little doubt that a new era in the chemotherapy of tuberculosis has dawned. Dr. Lincoln's observation, that very prolonged treatment with promizole is necessary for a good chemotherapeutic effect, is very important because one may be led astray if treatment is not continued long enough. This has recently been found by Dr. Feldman at the Mayo Clinic in an investigation with p-aminosalicylic acid on tuberculosis in the guinea pig. The first experiment with this substance showed absolutely no effect. Here treatment lasted for only forty-five days. When the experiment was repeated and treatment was continued for one hundred and fifteen days, a markedly favorable chemotherapeutic effect was obtained. I should also like to comment on Dr. Lincoln's very interesting and important results with combined therapy (streptomycin plus promizole) in tuberculous meningitis. Possibly this is carrying us back to the antiquated method of shotgun prescriptions. However, there is definite evidence on experimental infections in animals in favor of combined therapy. Dr. Lincoln mentioned M. I. Smith, who worked with promin and streptomycin in tuberculosis of the guinea pig. Recently, Feldman has found that a combination of streptomycin with p-aminosalicylic acid is more effective than either substance alone in experimental tuberculosis of the guinea pig. The same combination has been found highly effective in tuberculosis of the mouse. A possible explanation of the greater effectiveness of combined therapy may lie in a consideration of the data presented in a recent paper published in the *Journal of Bacteriology*. The author showed that the use of a mixture of streptomycin and sulfadiazine on a staphylococcus in vitro prevents the development of drug fastness

to either drug. Should this be confirmed and extended to other combinations it may offer a lead to the understanding of the beneficial effects of combined chemotherapy.

Dr. Perrin H. Long: I have been very much interested in Dr. Lincoln's paper tonight because the sulfones are quite active compounds, especially diamino diphenyl sulfone. Ten years ago it seemed to us that possibly they might be effective in subacute bacterial endocarditis. We treated four patients with this compound, and within a week we had retreated hastily from our position because immediately three patients developed acute hemolytic anemia. I would like to ask Dr. Lincoln a question. She said her patients developed cyanosis. This was the thing which should have tipped us off ten years ago, that we might get into difficulties because I have never seen people become quite so blue within twenty-four hours as the individuals to whom we gave diamino diphenyl sulfone. I want to ask first if these people became anemic from the promizole; secondly, in respect to the ataxia which these children developed, I have wondered what has happened to that particular sign after streptomycin was discontinued, because it is one of the most serious toxic reactions I think that has been noted. Certainly this has been true in the individuals in the veterans' group, and I am not sure that it is not as serious as the deafness which has been reported. After all, if you are deaf you can walk around and get out. Some of these ataxic individuals have learned to accommodate in the daytime, but don't dare go out in the dark because they will promptly fall down. Has the ataxia disappeared after treatment with streptomycin has been stopped?

Dr. Edith M. Lincoln: The ataxia bothered us a great deal at the beginning but the children seem to get used to it very easily. I think there is no doubt about it that there is damage to the nerves, actual destruction, but they adjust to it. This one little boy I told you about, the four year old, had to be sent home because he was so active, although he was so ataxic in the early stages that he was just weaving up and down the ward and would invariably fall. Before he left the hospital he could climb on top of the cubicles with just as much agility and dexterity as any child. That happened with all the patients that we had.

Dr. Francis F. Schwentker: I would like to know what happens to the tuberculin test after these children are under treatment.

Dr. Lincoln: The tuberculin tests in all cases that we have done have remained positive after promizole therapy.

Dr. Schwentker: I wondered whether there would be an increase or decrease in the size of the tuberculin reaction during therapy. Products of the killed tubercle bacilli might absorb the antibody and cause a decrease in the amount of reaction.

Dr. Lincoln: I am afraid we haven't tried that. In fact we are a little afraid of too many perifocal reactions around a cortical focus. We have done patch tests on children who have recovered. We expect, from the fact that the primary focus is visible for such a long time, that we shall have none which revert to negative.

Dr. Frederic B. Bang: I would like to ask Dr. Lincoln if any of her patients developed a red color of the urine.

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Dr. Lincoln: We have seen red color in the urine. This happens not infrequently and the cause has not been identified. In fact, the Parke-Davis directive warned us of this color and we have paid very little attention to it.

Dr. A. Murray Fisher: I would like to ask if the promizole is excreted in the spinal fluid, and also if the organisms have become resistant to streptomycin. Probably Dr. Lincoln hasn't had more than one or two relapses in which she has been able to obtain the organisms the second time. I wondered if they became resistant.

Dr. Lincoln: We have made no study on promizole excreted in the spinal fluid at all. Unfortunately, we haven't been able to do much study on resistance either because none of our children except one have relapsed and have had to be treated a second time. We rely in large part on the clinical reaction. I think if this small boy gets over the meningitis relapse after treatment for a second time, the chances are that his organisms are not resistant to streptomycin. None of the original cases of miliary tuberculosis relapsed, so we were not able to do resistance studies with promizole. We had one child who had a marked anemia. We thought he who had miliary tuberculosis and became deeply jaundiced. We also had one probably had tuberculosis of his liver and his jaundice was associated with that; whether it was due to that or to a toxic reaction to promizole I don't know. Two of them did have leucopenia which did not respond to folic acid or any other treatment, and which cleared spontaneously.

BOOK REVIEWS

Advances in Pediatrics, Vol. 2. Editors: S. Z. LEVINE, ALLAN M. BUTLER, L. EMMETT HOLT, JR., AND A. ASHLEY WEECH. Illus. 407 pp. \$6.75. *Inter-science Publishers, Inc., New York, New York*, 1947.

The volume of current medical literature has become so great that it has become practically impossible for one to keep abreast of the progress being made in many diverse fields. The need is being met by the publication of reviews or short monographs by authorities who are capable of reviewing, evaluating and bringing up to date the work on a particular subject. *Advances in Pediatrics* is published annually as a small volume with this objective. After the first issue in 1942 the publication was suspended during the war. Volume 2 has now appeared under new editorship. It fulfills its purpose excellently in presenting discussions by recognized authorities on pediatric subjects selected because of contemporary interest and importance. Its merits can be demonstrated best by listing the table of contents and authors:

Etiology of Congenital Malformations, by Josef Warkany

Acute Infectious Lymphocytosis, by Carl H. Smith

Role of Fluorine in Prevention and Treatment of Dental Caries, by H. Trendley Dean

The Treatment of Purulent Meningitis, by Hattie E. Alexander

Chemotherapy: Penicillin, Sulfonamides, Streptomycin and Tyrothricin, by Paul György and Henry F. Lee

Atypical Pneumonia, by John H. Dingle

Endocrine and Other Factors Determining the Growth of Children, by Nathan B. Talbot and Edna H. Sobel

Virus Diarrhea, by Katherine Dodd

Prematurity, by Harry H. Gordon

The Genesis of Physiologic Hyperbilirubinemia, by A. A. Weech

Prevention of Recurrences of Rheumatic Fever, by Ann G. Kuttner

The value of the volume is enhanced by abundant references which have been well indexed.

L. W.

Essentials of Pharmacology. By FRANCES K. OLDHAM, F. E. KELSEY, AND E. M. K. GEILING. Illus. 440 pp. \$5.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania*, 1947.

This book from the University of Chicago, Department of Pharmacology, presents in brief fashion the pharmacological principles of drugs in current clinical use. In general, each chapter consists of a short discussion of a group of drugs, usually with the inclusion of structural formulas, followed by a list of official preparations and a selected bibliography. The therapeutic concepts are in excellent conformity with modern formulations. Emphasis has been placed upon the most

recent pharmacological developments, so that the book is amazingly up to date. The inclusion of all the latest advances has been accomplished partly at the expense of older fundamental work, and the presentation is thus somewhat unbalanced. The same comment may be made about the bibliographies.

The defects of this volume are chiefly those consequent upon the attempt to compress a large field into a small book. The discussions of the individual drugs are insufficiently detailed to tell the reader how to apply the information to clinical situations. Hence, the appeal of this book will be largely to those who are relatively unfamiliar with modern pharmacology and who desire a rapid introduction. It could not serve as a medical school text except for the purposes of review, or as a supplement to more extensive but less contemporary works. The physician will find it useful only for bibliographic material and chemical structures of recently introduced drugs.

C. G. Z.

Expectant Motherhood. 2nd Edition revised. By NICHOLSON J. EASTMAN. 198 pp. \$1.50. Little Brown and Company, Boston, Massachusetts, 1947.

Advice books about pregnancy antedate the printed page, and if all were placed side by side a large library would result. Among the most widely read of the modern era is Dr. Eastman's *Expectant Motherhood*, first printed in 1940. During the succeeding seven years it was reprinted fifteen times and currently appears completely revised.

Expectant Motherhood is written for the intelligent layman. The author captivates the reader by his authoritative, sincere, simple style; a style devoid of window dressing. A great amount of valuable, practical information is packed into the small volume. If one were to offer any general criticism, he might take the author to task for the almost complete absence of historical data, a field in which the author has frequently demonstrated his special competence. The only excursion into medical history which he has allowed himself is a few paragraphs concerning the origin of obstetrical forceps. The reviewer feels that medical books written for the layman should not only present the essential facts but the historical evolution of their acquisition. Such a combination educates the public in an appreciation of modern scientific medicine, plus better understanding of its peripheral quackery.

Several new chapters have been added to the first edition. One of these is an excellent chapter on weight control in pregnancy with sample menus. "How to telephone your doctor" is an intriguing inclusion; its purpose is to make telephone consultations more efficient and satisfactory. Paragraphs on the much discussed Rh factor and on controversial caudal anaesthesia first appear in the revised edition.

In one instance Dr. Eastman allows faith in democracy to outweigh scientific critique. Because 84 per cent of seventy-five obstetrical authorities assume that smoking and inhaling twenty-five or more cigarettes daily have an unfavorable

effect on maternal health, he too implies this is the case. Yet neither he nor the other authorities offer scientific data to support this prejudice.

All in all *Expectant Motherhood* does an excellent job. Dr. Eastman makes a painstaking attempt to replace the subtle fears and folk 'misinformation of the pregnant woman and her kin by calm assurance and scientific knowledge. And he succeeds.

A. F. G.

Office Endocrinology. 3rd Ed. By ROBERT B. GREENBLATT. Illus. 303 pp. \$4.75. Charles C. Thomas, Springfield, Illinois, 1947.

This is a well-intentioned effort to bring together into a single small volume the confusing theories and the treatment of certain endocrine problems. The author has had extensive experience in this field, both in the laboratory and clinically. His interest has been centered primarily in female endocrinology, and the book should more properly be given this title. Except for a short section of 27 pages on male endocrinology and 32 pages on the action of hormones and the available commercial products, the entire book is concerned with gynecological endocrinology. The author, in his preface to this edition, emphasizes that it has been prepared "for the practicing physician with the usual office equipment and with the laboratory facilities afforded by the average community hospital."

Were the general practitioner to follow through chronologically the advice on diagnosis and treatment as outlined under the various headings, he would in many instances be hopelessly at sea and the patient's pocketbook would be severely depleted. No mention is made of the necessity of a diagnostic curettage and cervical biopsy before endocrine therapy is instituted in menometrorrhagia. In the conclusion of this chapter this advice is given in case the woman is over 36 years of age. Far too many early malignancies are missed now because the pharmaceutically advised general practitioner resorts to shots before he performs a pelvic examination. The physician who performs suction curettage "30 or 40 days after the last menstrual period" as advised in case of amenorrhea may be considerably chagrined when he studies the tissue sections. His suggestion to use testosterone in bleeding associated with myomas of the uterus can only be applicable in the very rare instance. In Chapter 16 the shortcomings of anterior pituitary extracts, equine gonadotropins, and chorionic gonadotropins as producers of ovulation in the human ovary are stressed, yet he strongly advocates their use in the chapter "The Management of Sterility". Leucoplakia of the vulva is not mentioned in the chapter on Pruritis Vulvae.

The chapters on the various hormones, their physiologic properties and their commercial names and dosages are especially informative and useful. He is particularly cautious in the part on male endocrinology, and for this reason the advised treatments, though in many instances controversial, are more rational.

The problems of obesity and thyroid disease are inadequately treated, whereas the less common problems of sexual libido and sexual infantilism in the female

are extensively and well covered. There is a chapter entitled "Endocrine Head-aches", which is diffuse and confusing, and might well have been omitted.

Clinical endocrinology is still an infant, and the shortcomings of this book are less the fault of the author than the inherent impossibility of the task he has set for himself. Many chapters contain material too controversial or poorly founded to be included in a working handbook for the indiscriminating, busy practitioner, who, in the words of the author's preface, "cannot keep up with rapid advances in the field nor is (he) interested in the polemics of why and wherefore". Inevitably this volume will encourage the practitioner of this description in the indiscriminate use of potent endocrine agents, though this is not the intent of the author.

R. B. S.
B. F. J.

Osteotomy of the Long Bones. By HENRY MILCH. Illus. 294 pp. \$6.75.
Charles C. Thomas, Springfield, Illinois, 1947.

Dr. Milch has done an excellent piece of work on a rather special aspect of orthopedic surgery. Following an interesting historical view of osteotomy, he develops his views on its objective and proceeds to describe lineal, tortional, transpositional, and angulational osteotomies of the long bones, such as the forearm, humerus, tibia, and fibula which he considers as straight bones because their axes are essentially in one plane.

He develops the osteotomy of the femur as a special feature in the second part of this book because of "that particular axial displacement of the femur which occurs in the coronal plane". There is much very valuable information in this concise work, and the charts of expected growth in children are especially valuable. He has applied a good deal of intelligent thought and wide experience. His views and methods will not, of course, gain unanimous approval, but his work is definitely worthwhile. "Osteotomy of the Long Bones" should be a valuable aid to the surgeon who must consider equalization of length or correction of deformity or dysfunction.

W. R. F.

Physiology of Man in the Desert. By E. F. ADOLPH AND ASSOCIATES. Illus. 357 pp. \$6.50. Interscience Publishers, Inc., New York, New York, 1947.

The exigency of the war period turned the efforts of many investigators into the field of environmental physiology. This book reports the extensive investigations of the University of Rochester Desert Unit made during four years of arduous field and laboratory study of man's response to heat. There is presented a wealth of data, much of it graphically, on heat exchange, sweat formation, water turnover, blood constituents, urinary salt and water excretion, and circulatory responses to heat exposure. Quantitative measurements of water deficit are correlated with clinical manifestations. Important observations are recorded relative to the mechanism of thirst. Much of the data have led to the formulation of useful rules for the maintenance of life and maximum efficiency in desert environ-

ments. There are also many observations on over-all water metabolism which may be applied directly to related clinical problems.

Dr. Adolph and his collaborators have written a most entertaining and valuable account of a very dry subject. The numerous charts and maps add great clarity to the presentation. The purpose served by the photographs is obscure.

J. L. L., Jr.

Treatment of Diabetes Mellitus, The. 8th Ed. By ELLIOTT P. JOSLIN, HOWARD F. ROOT, PRISCILLA WHITE, ALEXANDER MARBLE, AND C. CABELL BAILEY. Illus. 861 pp. \$10.00. *Lea & Febiger, Philadelphia, Pennsylvania*, 1946.

This standard text in the field of diabetes has been brought up to date by Joslin and his coworkers. The title is somewhat misleading since a large part of the book is concerned with etiology, diagnosis, pathology, and various complications of diabetes. The chapter on experimental diabetes produced by alloxan is well written.

There are numerous minor errors as is inevitable in so extensive a work. For example, on page 716, in discussing Cori's recent experimental observation of pituitary extract antagonism to insulin, pituitrin is referred to when, of course, anterior pituitary extract is meant; on page 153 the statement about blood galactose curve following oral administration of sugar is confusing; there might be disagreement with the statement on page 305 that normally the serum phosphorus is between 2 and 3 mg. per 100 cc.—but these are relatively minor points.

This book has long been of great value as a reference text. A wealth of material is covered, and numerous references are accurately recorded. Most of the chapters are well written and easy to read. The authors deserve much credit for the painstaking manner in which they have covered the field.

H. F. K., Jr.

Clinical Neuro-Ophthalmology. By FRANK B. WALSH. Illus. 1532 pp. \$15.00. *The Williams and Wilkins Company, Baltimore, Maryland*, 1947.

This volume by Doctor Walsh represents years of profound study of clinical neuro-ophthalmology. It is a good deal more than a textbook—it is practically a compendium of information. The illustrations throughout are excellent. The text is clear and the references adequately cover the field.

The domain of the book extends, however, considerably beyond the range of the usual clinical neuro-ophthalmology. There are chapters on infections, ocular syphilis, familial degenerative diseases, toxic and metabolic diseases, etc. It might have been somewhat easier for the reader had this splendid book been separated into two volumes, one being limited to neuro-ophthalmology and the other to ophthalmic conditions which at times produce neuro-ophthalmological symptoms. The book is saved from confusion by its splendid index which enables the reader to follow the subject rapidly to its conclusion through its various ramifications.

This book belongs in the library of every ophthalmologist and neurologist. It is a splendid piece of work.

A. C. W.

Gifford's Textbook of Ophthalmology, 4th Ed. By FRANCIS H. ADLER. Illus 512 pp. \$6.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

This book by Doctor Adler purports to be a re-editing of the late Doctor Gifford's well-known textbook. As a matter of fact, it is far more than that. Virtually, it is a completely new textbook on ophthalmology, especially designed for students, embodying somewhat the general form of Doctor Gifford's book. This book is not only an admirable volume for students or beginners in ophthalmology, but will also be definitely helpful to internists and physicians, who seek a clear-cut description and explanation for the various ocular anomalies which they constantly encounter.

The description of medical ophthalmology throughout is remarkably good, clear and comprehensive. At the end of each chapter is a small but well selected bibliography which will enable the student to explore further any subject in which he may be especially interested.

If any criticism is to be made of this book, it might be the undue attention devoted to disturbances in ocular motility. However, the author is especially interested in this field, in which he has made outstanding advances.

This is not only a good book; it is probably the best small textbook on modern ophthalmology.

A. C. W.

Textbook of Bacteriology, A. By THURMAN B. RICE. 4th Ed. Illus. 603 pp. \$6.50. W. B. Saunders Co., Philadelphia, Pennsylvania, 1947.

According to the author's prefaces this book "is not intended to serve as a manual for the determination of the identity of species. It is intended as a basis for the understanding of disease, to the end that diagnosis, prognosis, treatment and general management may be as efficient as is possible." "The technical processes described in detail are only those which may be done by the practicing physician, limited as he is in time, material, and equipment. When more is needed, he is instructed only in the manner of taking the sample, of sending it to the laboratory, and of interpreting the results." It is the opinion of the reviewer that the object stated in the second quoted sentence is unattainable under the limitations stated in the other sentences quoted. It is our opinion that a textbook should not be merely a brief outline of what the student is expected to learn and retain, but a book which can be used for reference during subsequent studies and practice.

The author seldom refers to original work and gives practically no references to literature. In justification he states—"It has been our experience that students do not use the bibliographies given in textbooks." Our experience has been otherwise. Different attitudes on the part of students can be cultivated by differences in teaching. In the long run mere epiricism may be less *practical* than habits of scholarship.

We would regard the book as inadequate for the minimum requirements of a medical student.

J. H. B.

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- P-Q-R-S-T: A Guide to Electrocardiogram Interpretation*. By JOSEPH E. F. RISEMAN, 2nd Ed. Illus. 84 pp. \$3.50. The MacMillan Company, New York, New York, 1947.
- Pharmaceutical Laboratory Manual*. By R. A. KUEVER. 289 pp. \$2.75. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
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- Unipolar Lead Electrocardiography*. By EMANUEL GOLDBERGER. Illus. 182 pp. \$4.00. Lea & Febiger, Philadelphia, Pennsylvania, 1947.

EFFECT OF ATROPINE AND ESTROGENS ON INTRAOCULAR UTERINE TRANSPLANTS IN THE RABBIT

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The transplantation of organs or parts of organs to the anterior chamber of the eye provides unique opportunities for the study of the morphologic manifestation of physiological responses, since the transparency of the cornea allows observation without anesthesia of the structures which implant in the anterior chamber. The uterus undergoes alterations under physiological conditions which are particularly suitable for such observation. The endometrium manifests characteristic changes in color and size in response to hormone deprivation and estrogen and progesterone stimulation, while contractions of the myometrium produce discernible motion.

In the course of a study of the mode of action of prostigmin as an emmenagogue it appeared desirable to reinvestigate the supposed cholinergic mechanism of estrogen action on the endometrium of the rabbit, taking advantage of the physiologic conditions available with intraocular transplants. The results of this investigation cast serious doubt on the hitherto accepted conclusion that estrogens produce vasodilatation and stasis of the endometrial vessels by local production of acetylcholine.

MATERIALS AND METHODS

The thirty-one rabbits employed in these experiments were standard laboratory animals of no particular strain, purchased from a local dealer and subsequently kept in individual cages on a laboratory diet. They were all in good health with the exception of a few animals which were sacrificed because of chronic suppurative otitis media secondary to tick infestation of the external ear. No animal was

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mated during the experimental period and none became pseudopregnant except as noted. Only autotransplants were made.

Operations were performed under anesthesia with intravenous barbiturates or ether by cone, neither of which was entirely satisfactory. The abdomen was opened and the ovaries inspected. A small segment of uterus, usually from the left side, was then excised with scissors. No effort at hemostasis was made, simple closure of the abdominal wound being found adequate. In the earlier experiments this segment was placed on a gauze sponge and opened lengthwise. It was then spread out on its serosal surface and with fine curved scissors attempts were made to remove small segments of endometrium from the myometrium. In many cases the wall of the uterus thus spread out was so thin that most fragments included the entire thickness of the wall. By trial and error a few partial-thickness slices could be obtained. Later in the series, at the suggestion of Dr. J. Eldredge Markee, the opened uteri were laid on the index finger and then cut with scissors, but this technique did not increase the rate of successful grafting. In an attempt to solve this problem, several animals were injected with human pregnancy urine and operated upon on the 12th day, at which time a thick uterine wall was present. It was then simple to excise small fragments of endometrium and transplant them, but as the pseudopregnancy receded these grafts gradually vanished, and none reappeared during subsequent hormonal stimulation. Considerable attention was paid to this technical problem of obtaining endometrium without myometrium for grafting because in the vast majority of grafts the physiologic activity was dominated by myometrial contractions, rendering the grafts unsuitable for the particular purposes of these experiments. The problem was not solved, and the successful endometrial grafts were obtained by apparently fortuitous circumstances which could not be reproduced. Every successful graft at one time or another manifested myometrial activity.

The eyes were prepared for operation by instillation of saturated boric acid solution. It was found that ether, which lowered the intraocular tension, rendered the operation more difficult, while the intravenous barbiturates did not. Preoperative atropine administration also caused technical difficulties. In the majority of cases the

right eye only was used, as described below, and two grafts were placed, but in some experiments both eyes were employed. The upper lid and nictitating membrane were retracted by an assistant. The cornea was incised with an iridectomy knife at a position corresponding to about 2 o'clock on a dial and about 2 mm. from the corneoscleral margin, the blade being held parallel with the margin and inserted with a rapid stabbing motion. Occasionally the underlying iris was lacerated by the knife blade, but serious hemorrhage resulted only when the incision had been made too close to the corneoscleral margin. Such hemorrhage usually resulted in failure of the grafts. The corneal incision was then extended slightly inferiorly with fine scissors. The fragment of uterus was grasped with a fine toothed forceps and inserted into the anterior chamber. Because of the small size of the grafts, no effort could be made to orient the uterine tissue layers within the anterior chamber. Once inside the chamber, the uterine fragments were manipulated into position at 10 and 12 o'clock in the corneoidridial angle by use of counter-pressure against the cornea with the rounded end of a scalpel handle or forceps. The cornea was not sutured.

Postoperatively, considerable hemorrhage was observed in only a few animals, and in these the grafts were usually not successful, due to scarring of the graft and corneal opacity. Most grafts were hemorrhagic for the first 5 to 7 days. Several animals suffered infections of the cornea. A few of these were treated with instillations of sulfathiazole ointment in the conjunctival space, with marked resolution of the superficial keratitis and conjunctivitis, but most of these animals had a residual haziness of the cornea. It was noted that however damaged a graft might look one or two weeks after operation, after one month it might eventuate as a successful take with little or no corneal reaction. The cornea healed promptly in all but the few cases in which pieces of graft herniated into the corneal defect. In these there was marked infection. Once a successful take was established the animal required no further special care and, although the operated eyes appeared to have defective vision this did not seriously alter the animals' general or mating behavior. The conclusion that vision was defective in the operated eyes is based on the observations that these animals were invariably found in their cages sitting with

the unoperated eye toward the room, and that they usually had suffered subluxation of the lens.

For observation, the animals were strapped to boards in the supine position and covered with a towel to conserve body heat. The head was held in position and the eyelids retracted by the observer's left hand. A large binocular dissecting microscope to which a lamp with a condensing lens had been attached was then swung into place over the eye and observations were begun. Continuous records were kept of all experiments with a watch and stop-watch, so that the time of onset and duration of any changes could be noted. Injections were given in the ear veins, which were usually prepared in advance by shaving the ear. These caused no fright response from the animals unless a dull needle was employed or an extravasation occurred. The majority of animals quieted down after the first few minutes of observations and remained quiet and unresisting for the course of the experiments, some of which lasted as long as 150 minutes. On occasion violent struggling precluded observation for brief periods of time, but if this became too marked the experiment was discontinued.

In the course of this study, all injections were made by the intravenous route. Atropine sulphate was employed in 1% aqueous solution. Amniotin in corn oil solution² was given in amounts ranging from 150 to 500 i.u. 0.01 mgm. of stilbestrol, estimated to be equivalent to 250 i.u. of estrone, was given in corn oil solution. 220 i.u. of sodium estrone³ was given in aqueous solution. All injections were administered by an assistant, from a tuberculin syringe.

Preparations for microscopic study were made by excising the eyes immediately after the animal was sacrificed and immersing them in Bouin's fluid. With a 26-gage needle Bouin's fluid was mixed with the vitreous humor by injecting small amounts of the fixative and withdrawing part of the vitreous. This was repeated until a high concentration of Bouin's fluid was obtained, without at any time collapsing the globe. The eye was then turned over and Bouin's fluid similarly

² The Amniotin employed in this study was generously provided by E. R. Squibb and Sons Inc.

³ The estrone (Menformon) employed in this study was generously provided by Roche Organon Inc. as an aqueous solution of the sodium salt in a concentration of 1400 i.u. per cc.

injected into the anterior chamber. After 24 hours a large window was cut in the posterior hemisphere of the globe. This technique insures optimal fixation and maintenance of anatomical relationships. The blocks were imbedded in celloidin and paraffin for several weeks so as to minimize fragmenting of the cornea and then serially sectioned at 15 μ .

APPEARANCE OF GRAFTS

The particular shape assumed by the graft is not in any obvious way related to the shape of the tissue fragment implanted in the anterior chamber. Grafts implant in the iris or on the cornea or both. When implantation on the iris has occurred, contraction of the iris produces slight changes in the grafts which do not interfere with observations. In all cases many blood vessels descend over the surface of the sclera toward the corneoscleral border adjacent to the graft, where the majority of them apparently penetrate the globe to supply the graft. Some smaller branches of capillary size extend in a network over the surface of the cornea in the region of the graft and, if this is near the corneal incision, to the corneal scar as well. Vessels are occasionally observed coursing in the iris toward the graft, but the bulk of the blood supply of the grafts appears to come through the scleral vessels rather than through the blood supply of the iris.

The grafts themselves are made up of delicate looking tissue of an orange to pink color, whose free borders are clean and sharply defined. Many of the vessels can be clearly seen either on the surface or in the substance of the graft. In some grafts arterioles and venules are present but most vessels are of capillary size. In one large graft it is possible to observe the mouths of endometrial glands on the inferior free border. Fragments of uveal pigment are imbedded in some grafts and this pigment is often heaped up at the margins of the iridial attachment. Some of these features can be seen in Figure 1.

As can be seen in Figure 2, the endometrium maintains its histological integrity in the anterior chamber. The transition from surface epithelium of the endometrium to iridial or corneal epithelium is a gradual one and the stroma of the iris and endometrium tend to blend together at the margins of the grafts. Endometrial cysts of microscopic size are frequent. The myometrium present in the graft is

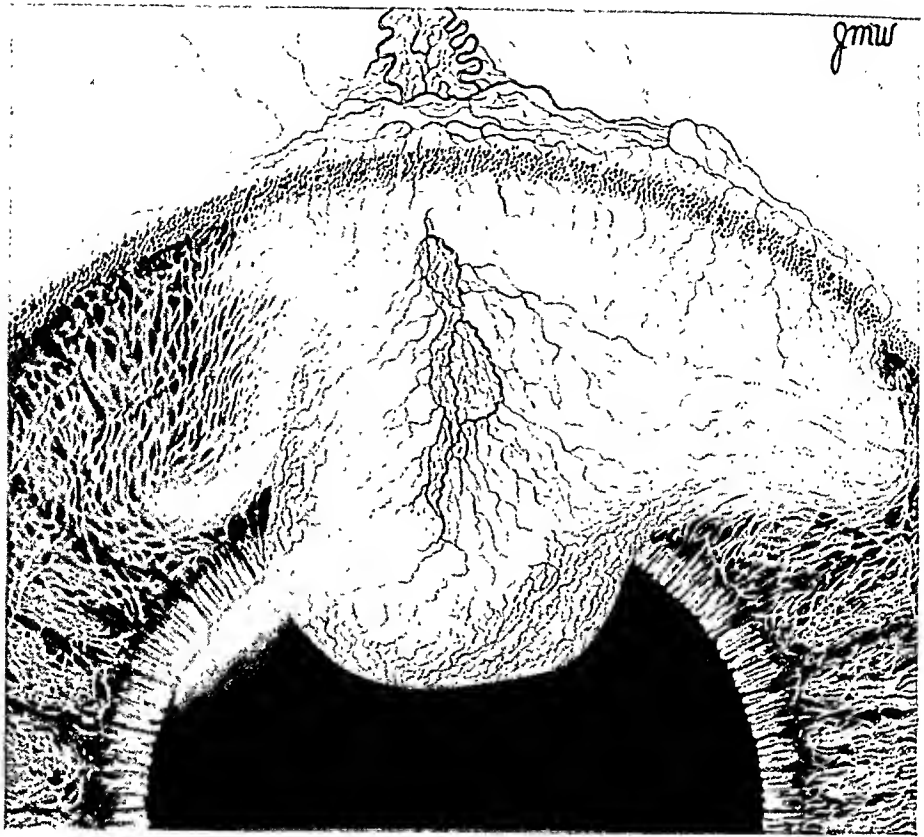


FIG. 1. DRAWING SHOWING PORTION OF A RABBIT'S EYE WITH A UTERINE GRAFT IMPLANTED IN THE ANTERIOR CHAMBER

The cornea is unaltered except for the whitish area in the cornea at the extreme left. This is the healed scar of the incision made for the insertion of the tissues for grafting. The largest visible portion of the graft, which is orange in color, is applied to the internal surface of the cornea. The small rose-red area at the lower right side of the graft is the only visible surface free in the anterior chamber. At the right and left sides of the graft there are areas of fibrous scarring, yellow in color, which form part of its attachment to the iris. These scars distort the iris.

The large vessels which run toward the graft in the superficial layers of the sclera give off numerous small lateral channels which run along the corneoscleral border. These in turn give rise to smaller branches which run into the cornea over the region of the graft.

In the center of the graft lie its main vessels, an anastomosing group which forms a rough figure-of-eight. These vessels travel in the superficial layers of the graft almost immediately beneath the cornea. They give rise to many smaller branches. Many of the capillaries visible in the graft do not appear to arise from this main group. Rhythmical vasoconstriction can be observed in the vessels of the graft itself in the absence of any alterations in the vessels of the sclera, cornea or iris.

always placed at the main site of attachment and blends into the substance of the iris by fibrous union. Myometrial bundles are readily identifiable and readily distinguished from the iridial musculature by their position within the graft. The major blood vessels supplying the graft course through these bundles of myometrial fibers, as demonstrated in Figure 3. In some cases the bulk of the graft is found behind the iris where it could not have been observed in the intact animal.

ACTIVITY OF THE GRAFTS

In the process of observation for brief periods of time, only muscular and vascular activity can be observed. The growth processes of endometrium are not discernible.

Muscular activity can be identified by the occurrence of visible contractions. Not infrequently these are of very slight amplitude and can be seen only when the graft is viewed from an advantageous angle. As has been noted, in most cases the vascular supply of the graft enters through the muscle bundles and contractions are followed by paling of the remainder of the graft. This paling proceeds from the area of attachment of the myometrial fibers to the iris to the rest of the graft, making it appear as if the arterial supply were completely occluded but the venous drainage at least partially free so that all blood rapidly empties out of the graft. These muscle contractions last from 20 to 45 seconds and the paling of the graft a corresponding length of time. Once the behavior of a particular graft becomes familiar to the observer, it is possible to identify contractions without observing the movement which they produce.

Vascular activity also occurs spontaneously in mature animals with intact ovaries, in the absence of heat or mating. Every 45 to 120 seconds the vessels of the graft fade out of sight for periods of 5 to 20 seconds. The fading is not accompanied by any visible muscular contraction. This paling does not appear to be initiated in any particular area, nor does it affect all the capillaries in the graft completely, so that there is always some residual orange-pink ground color remaining in the graft. During the phase without vasoconstriction, there is no fluctuation in the caliber or number of patent vessels, so that in fact there is no blush. The rhythmical vasoconstrictions continue at a rate of about 30 to 60 per hour for as long as observation is maintained.

FIG. 2

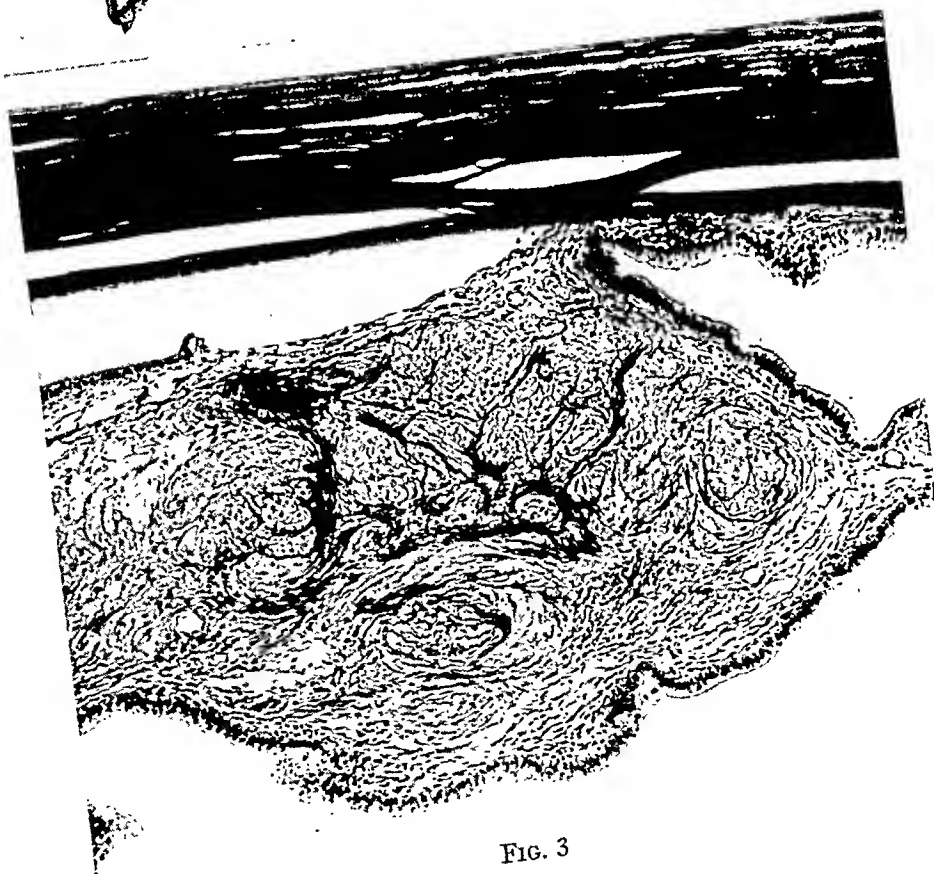
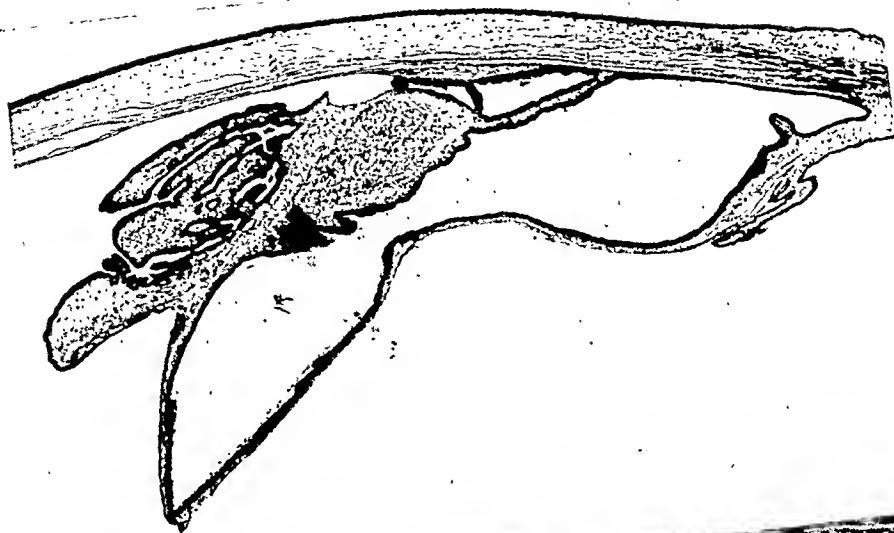


FIG. 3

The phenomenon involves only the intrinsic vessels of the graft and does not affect iridial or scleral vessels related to the graft.

In response to fright, the vessels of the graft undergo intense vasoconstriction which results in a bloodless deathly white appearance in the graft. This persists for 10 to 25 seconds and also affects, to a lesser degree, the vessels at the corneoscleral margins. It is usually accompanied by a muscular contraction whose characteristics differ in no way from those described for the spontaneously occurring contractions except that the fright contraction is occasionally more intense or more prolonged. There is no rhythmical pattern to the vasoconstrictions of fright. On the basis of these characteristics they can readily be distinguished from the rhythmical vasoconstrictions.

In certain grafts, both muscular contractions and rhythmical vasoconstrictions are present. Often the myometrial phenomena occur at 5 minute intervals and the more frequently occurring vasoconstrictions can be identified. Of course, if a muscle contraction immediately

FIG. 2. CROSS SECTION OF A UTERINE GRAFT IN THE ANTERIOR CHAMBER OF ANOTHER RABBIT'S EYE (R5-200), STAINED WITH HEMATOXYLIN, EOSIN AND PHLOXINE AND ENLARGED 20 TIMES

The margin of the sclera is at the right. The cornea runs across the remainder of the upper portion of the photograph. Two corneoscleral vessels can be seen just under the bulbar conjunctiva at the right. The low cuboidal epithelium of the iris covers the entire posterior border of the iris and the graft and blends into the darker staining ciliated columnar epithelium of the uterine graft at the left. This uterine epithelium forms typical endometrial glands supported by endometrial stroma in the portion of the graft to the left. It also forms one large and two small cystic spaces. Just to the left of the center of the photograph there is a large group of myometrial bundles through which the main arterial channels of the graft pass. These may be seen more clearly in Figure 3.

FIG. 3. VAN GIESON STAIN OF A SECTION (R5-204) ADJACENT TO THE ONE IN FIGURE 2, ENLARGED 100 TIMES

The deeply staining fibers of the cornea run across the top of the photograph. The uterine epithelium which lines the cystic areas is columnar and in some places its ciliated border can be made out. In the center of the photograph the brown staining smooth muscle fibers of the myometrium form bundles which encircle at least three small arteries. Several small veins are present at the periphery of the group of myometrial bundles, encased in fibrous tissue. These vessels form the vasculature of the uterine graft.

precedes vasoconstriction there is no way, with present techniques, of being certain that the latter has occurred. For that reason, in keeping the protocols of these experiments, all paling of the graft which was preceded by visible myometrial contraction was regarded as a muscle response only.

A few grafts exhibit vasoconstrictions at one time and myometrial responses at another. In addition, when two grafts are present in the same eye, one may exhibit muscle contractions and the other vasoconstrictions. It is therefore necessary to observe each graft carefully during a control period to determine its physiologic status before administering any substance.

RESULTS

Effect of Atropine. The effect of atropine is entirely dependent upon the type of physiologic activity present in the graft at the time of administration. In the doses employed, atropine acts to slow or halt endometrial vasoconstrictions, whereas it has no predictable effect on myometrial contractions. As can be noted in Table I, there is no direct correlation between dose and the degree or duration of effect of atropine on vasoconstrictions, except that the larger doses appear to be slightly more effective. The greatest and least percentage changes were obtained with the same dose. The effect in general begins to wear off within ten minutes of administration of the drug, but complete cessation of vascular activity for periods as long as 45 minutes is observed.

During the periods when vasoconstrictions are halted, the vessels have an engorged appearance but additional vascular channels do not appear to open, and it may well be that the appearance of engorgement is simply due to the striking contrast of inactivity with the periodic constrictions which are observed before atropine. The full effect of estrogens is strikingly different.

The blood vessels of grafts which do not manifest either vascular or myometrial activity are not affected by atropine.

The effect of atropine on myometrial contractions in intraocular transplants is quite unpredictable. As may be seen in Table II, it may produce decrease or increase in the rate of contractions. These effects are not dependent upon the size of the dose. Except for changes

in the rate of contraction, the appearance of the grafts is in no wise altered, and the vascular phenomena described in grafts manifesting rhythmical vasoconstriction are not observed.

Effect of Estrogens. There are no significant differences among the estrogens employed in their effects on vasoconstrictions and contractions. Shortly after the administration of the hormone, the vasoconstrictions begin to decrease in frequency until at the end of one hour

TABLE I

Effect of Atropine on Endometrial Vasoconstrictions in Intraocular Transplants

DOSE OF ATROPINE	NO. OF RABBIT	DATE	CONTROL—15 MIN. PERIODS*	RESULT—CONSTRICTIONS PER HOUR*	
			Constrictions per hour	First 15 mins.	Second 15 mins.
<i>mgm.</i>					
0.6	41	Aug. 13	32	8	8
	41	Aug. 14	36	12	12
	41	Aug. 19	32	8	8
	41	Aug. 20	40	4	16
2.0	41	Aug. 21	44	20	
	41	Aug. 23	56	0	12
	39	Sep. 10	48	4	28
	39	Oct. 18	56	16	44
	39	Oct. 24	60	8	28
4.0-6.0	41	Sep. 13	48	4	20
	5	Oct. 21	24	0	0
	5	Oct. 15	36	0	
	39	Sep. 19	60	0	
	39	Oct. 9	72	20	52
	39	Oct. 29	52	24	

* In each experiment the data given represent consecutive periods during continuous observation.

vascular activity has virtually ceased. With amniotin, this effect begins to wear off after one and one-half hours, but with sodium estrone and stilbestrol it persists for as long as three hours. This effect is presented in graphic form in Chart IA. Coincidentally with this slowing of rhythmical vasoconstrictions there occurs a gradual dilatation of the vessels and the appearance of new channels, so that at the peak of hormone effect the graft has an intense rose-red color which provides a

striking contrast to the unaltered appearance of the vessels of the adjacent corneoscleral border. This engorgement is observed, in much less striking form, in grafts which have not manifested endometrial vasoconstrictions during the control period. Estrogens do not appear to influence the rate, duration or intensity of myometrial contractions during the course of these acute experiments.

TABLE II

Effect of Atropine on Myometrial Contractions in Intraocular Transplants

DOSE OF ATROPINE	NO. OF RABBIT	DATE	CONTROL—15 MIN. PERIOD*	RESULT—CONTRACTIONS PER HOUR*	
			Contractions per hour	First 15 mins.	Second 15 mins.
<i>mgm.</i>					
0.6	14	Aug. 14	36	28	
	14	Aug. 20	28	28	
	31	Oct. 31	60	32	40
2.0	14	Aug. 26	40	36	
	31	Oct. 18	36	16	24
	39	Oct. 24	8	16	8
	41	Oct. 22	24	0	4
	5	Oct. 30	40	28	32
4.0	5	Oct. 21	16	0	4
	12	Oct. 21	16	12	20
	14	Aug. 28	32	44	24
	31	Oct. 24	32	24	36
	35	Oct. 24	44	44	
	41	Oct. 30	40	12	28
6.0	14	Oct. 4	24	28	24
	41	Oct. 2	20	16	
	12	Oct. 30	24	16	12
	35	Oct. 31	40	32	44

* In each experiment the data given represent consecutive periods during continuous observation.

Influence of Atropine on the Effect of Estrogens. The administration of large (4 to 6 mgm.) doses of atropine prior to, simulatenously with or subsequently to the administration of estrogens in no way alters the effect of the latter on endometrial vasoconstrictions. This relationship may be seen in Chart IB, which illustrates three experiments in which atropine produces its expected effect of slowing vasoconstrictions

CHART IA

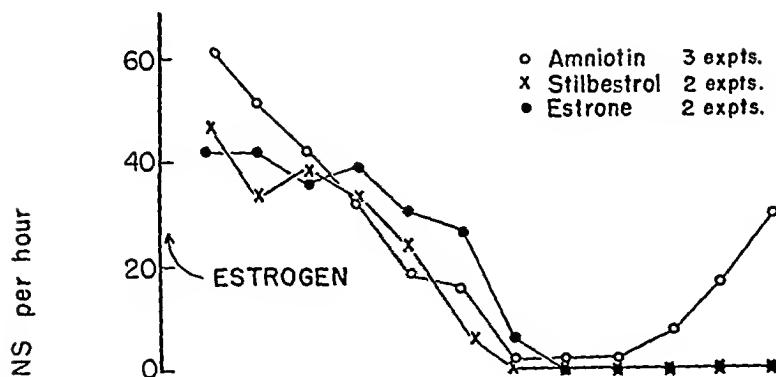


CHART IB

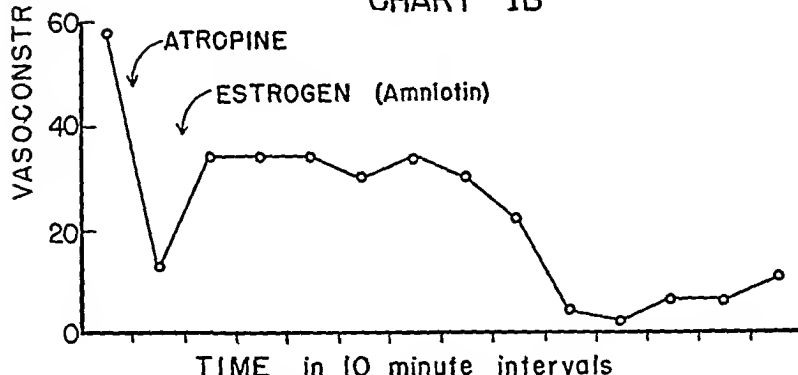


CHART I. Chart IA demonstrates the delayed effect of three estrogens in reducing the rate of endometrial vasoconstrictions. Chart IB demonstrates the immediate reduction in the rate of vasoconstrictions due to atropine. Administration of estrogens intravenously ten minutes after atropine then produces the same delayed effect seen in IA, at about the same time. Beginning recovery from the atropine can be observed in the second ten minute period in IB. Administration of atropine alone does not produce the delayed effect demonstrated in IB.

The dose of estrogens employed in this group of experiments varies from 150 to 500 i.u. of estrone. The majority are about 250 i.u. (0.01 mgm. of stilbestrol). The dose of atropine is 4 to 6 mgm. The arrows indicate the time of intravenous administration of the drugs. The abscissa is divided into consecutive 10 minute periods. Each point plotted represents the average number of vasoconstrictions observed in each time period, expressed as vasoconstrictions per hour, as indicated on the ordinates.

and, while this is at its maximum, amniotin is given. As the atropine effect is waning, estrogen effect is waxing, thereby producing a plateau

for a brief period. The cessation of rhythmical activity and the vasodilatation which mark estrogen effect appear at about the time they would be expected to do so without atropine effect. The continuing physiologic action of atropine may be noted by the mydriasis which persists for 18 to 48 hours in these animals.

DISCUSSION

In the course of this investigation, many of the observations of Markee (3, 4, 5) and Neumann (6, 7) on the physiology of intraocular endometrial transplants in the rabbit have been confirmed.⁴ Perhaps the most frequently discussed of these is the "blush and blanch" phenomenon. This rhythmical paling of the endometrium in the eye grafts also occurs in primates, and since menstruation is preceded by a period of ischemia a relationship between the "blush and blanch" and the menstrual process has been suggested. "Blush and blanch" is an unfortunate epithet for the phenomenon because there is no phase of vasodilatation. On the other hand, rhythmical paling describes what is actually observed.

The entire blood supply of the endometrium passes through the myometrium. It is obvious that contractions of uterine muscle will therefore reduce endometrial blood supply when the pressure they exert exceeds the diastolic pressure within the myometrial arteries. Nevertheless, in the present study rhythmical paling of endometrium has been observed in the absence of visible myometrial contraction. It is possible that this paling is due to changes in myometrial tonus without perceptible contraction but the remarkable cessation of activity in the endometrium in response to atropine makes this unlikely. The phenomenon appears to be intrinsic in the endometrial vessels and has therefore been designated as rhythmical vasoconstriction. In the intact uterus, rhythmical paling is due to rhythmical myometrial contraction and to rhythmical vasoconstriction. The concept of endometrial activity as separate from myometrial activity may be of value in the experimental analysis of events, as it has been

⁴ See also Jacobsen, E. M.: Response of intraocular endometrial implants to estrogens⁵ in the female rabbit. *Endocrinology*, 34: 376-388 (1944), for a discussion of the effects of estrogens over longer periods of time than those considered here.

here, but caution must be exercised in applying it to uterine phenomena in the living animal.

The present observations of the unpredictable effect of atropine on myometrial behavior confirm previous studies of uterine motility in the rabbit. They also indicate that intraocular transplantation makes possible the observation of muscle behavior without the imposition of work upon the muscle. In conventional physiological experiments, muscle exerts force against a load in the process of making records. In the anterior chamber no such load exists, yet accurate qualitative observation is simple. In this laboratory, the oxytocic effects of certain antihistaminic substances were initially noted in intraocular transplants and later confirmed by conventional techniques (2).

The failure of atropine to inhibit the action of estrogen upon endometrial vasoconstrictions appears to negate the so-called cholinergic concept of estrogenic effect on the endometrium. Pompen in 1933 (8), observing the intact rabbit uterus through a window in the abdominal wall, noted that the administration of small amounts of estrogen, in the same dose range as those used in the present study, caused vascular stasis and dilatation. He further noted that this effect was prevented by the prior administration of atropine intravenously. Reynolds, in a series of publications (9, 10, 11, 12), analyzed the changes in the acetylcholine content of the castrate rabbit uterus following the administration of small amounts of various estrogens, and found that there was a marked rise in acetylcholine after every estrogen except stilbestrol. Emmens' (1) subsequent inability to confirm this may have been due to differences in technique of determining acetylcholine. Soskin, Wachtel and Hechter (13) later assumed, on the basis of Reynolds' findings for the whole uterus, that estrogen effect on endometrium was due to acetylcholine. Pompen, however, was not observing endometrium, nor did Reynolds assay the layers of the uterus separately. In the present study it has been found that the effect of atropine on endometrium is identical with that of estrogens in halting rhythmical vasoconstrictions and in failing to affect myometrial contractions in any predictable manner. Atropine does not inhibit the effect of estrogens on endometrium, despite the fact that it is a powerful acetylcholine inhibitor. Furthermore, stilbestrol, which Reynolds found does not cause a rise in ace-

tylcholine content of the castrate rabbit uterus, produces an endometrial effect identical with that of sodium estrone and amniotin. It seems likely that Reynolds was measuring changes in acetylcholine in the myometrium only, especially since myometrium forms the bulk of the castrate rabbit uterus. Unless these effects are a species peculiarity of the rabbit, the acute effect of estrogen on the endometrium is not mediated by changes in endometrial acetylcholine.

SUMMARY

1. The physiological activity of uterine tissues can be observed in intraocular transplants. Endometrium maintains its histologic integrity in grafts and myometrium grows at the main site of attachment in bundles surrounding the vascular supply of the endometrium. Myometrial contractions can be identified by the motion and the occlusion of blood supply which they produce. The blood vessels of the endometrium undergo rhythmical vasoconstriction in non-pregnant mature animals.

2. In the rabbit, atropine causes a slowing or cessation of endometrial vasoconstrictions but does not predictably affect contractions of myometrium. Estrogens produce a slowing or cessation of endometrial vasoconstrictions and a dilatation of endometrial vessels but do not affect myometrial contractions. The administration of atropine does not alter the endometrial responses to estrogens regardless of time relationships.

3. These observations necessitate revision of the cholinergic concept of the effect of estrogens on the endometrium.

The histological sections were prepared by Mr. Joseph P. Drane and photographed by Mr. Chester Reather. The drawing was prepared by Mrs. Jeannette M. Whitehorn.

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A MULTI-CHANNEL STRAIN-GAGE TOKODYNAMOMETER: AN INSTRUMENT FOR STUDYING PATTERNS OF UTERINE CONTRACTIONS IN PREGNANT WOMEN¹

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1. REQUIREMENTS OF A TOKODYNAMOMETER

Uterine inertia is one of the chief problems facing the obstetrician today. It is a condition which is recognized only by its occurrence since there is nothing in a clinical history which presages its imminence. Uterine inertia, however, presents but one aspect of a general problem which confronts the obstetrician, namely, that of uterine dyskinesias during labor. These include the clinically recognized dystocias, contraction rings, and prolonged types of labor. The identification of these clinical entities is still a matter of clinical recognition as they occur. No physiological basis has yet been established for them. This is, accordingly, an important field for clinical and physiological investigation.

Perhaps the greatest lack in our knowledge of these conditions is that of a description of the basic patterns of uterine contractility in women before and during labor. A method is urgently needed which will enable us to visualize the origin and spread of the uterine contractions in order that we may associate different contraction types with different clinical conditions. This paper contains a description of an apparatus which makes this possible.

It is true that much is known concerning the comparative aspects of the activity of the uterus in labor. This subject was extensively reviewed eight years ago (Reynolds, 1939). There have been numerous studies of motility of the human uterus during labor, in addition to comparative studies. These are of three types: namely, (a) those concerned with direct measurements of intrauterine pressures (Schatz, 1871-72, Rucker, 1922, Woodbury, Hamilton and Torpin, 1938, and

¹ A preliminary note on the tokodynamometer appeared in Science for October 31, 1947 (Reynolds, Heard and Bruns).

others); (b) those concerned with measurement of the frequency of uterine contractions from the exterior of the abdominal wall by means of a single tokograph. All such units thus far used are so made that they record the activity at only one point. The Lorand tokograph employed extensively by Murphy (1946) is the tokograph most favored in recent years. The third method (c) is that of electrometric measurements of uterine action potentials. This method has not proved to be satisfactory in so far as the gravid uterus is concerned (Dill and Maiden, 1946).

The difficulty inherent in the foregoing methods is that they fail to yield information concerning the origin and spread of the uterine contractions. Instead, they give limited information concerning the expulsive force of the uterus or the frequency and duration of the contractions at a single point.

The ideal method would combine these features into one unit. The instrument described in this communication is the result of an attempt to design such an instrument. It is characterized by ease of operation and simultaneous multichannel electrical recording through the abdominal wall from three separate parts of the uterus. No photographic, ink-writing or kymographic procedure in the usual sense is employed. Instead, the instrument described below and shown in figure 1 records electrically on a single piece of moving paper the uterine activity during the latter months of pregnancy. The principle employed involves application of the strain-gage dynamometer to direct electrometric recording through frictionless writing. The key to this method is the specially devised pick-up which makes it possible to apply a strain-gage of known characteristics to the abdomen. Since three records are obtained on a single piece of paper, it is possible to measure simultaneously the characteristics of single contractions ((1) contraction-time, (2) relaxation-time, (3) contraction-rate, (4) force applied through the abdominal wall on the pick-up to the active element of the strain-gage), and to compare the time relations of any part of one contraction with the activity at either of the other two points simultaneously.

2. NAME OF RECORDING UNIT

The unit just described has been named a *tokodynamometer*. This word is taken from Websters' New Unabridged Dictionary. The

word is a compound one derived from the two following roots: *toko*, meaning "pertaining to birth"; *dynamometer*, indicating "measurement of force." While the complete descriptive name of multiple channel tokodynamometer is cumbersome, it lends itself to abbreviation by the convenient letters TKD. The records obtained on the TKD are tokodynagraphs, or TKGs.

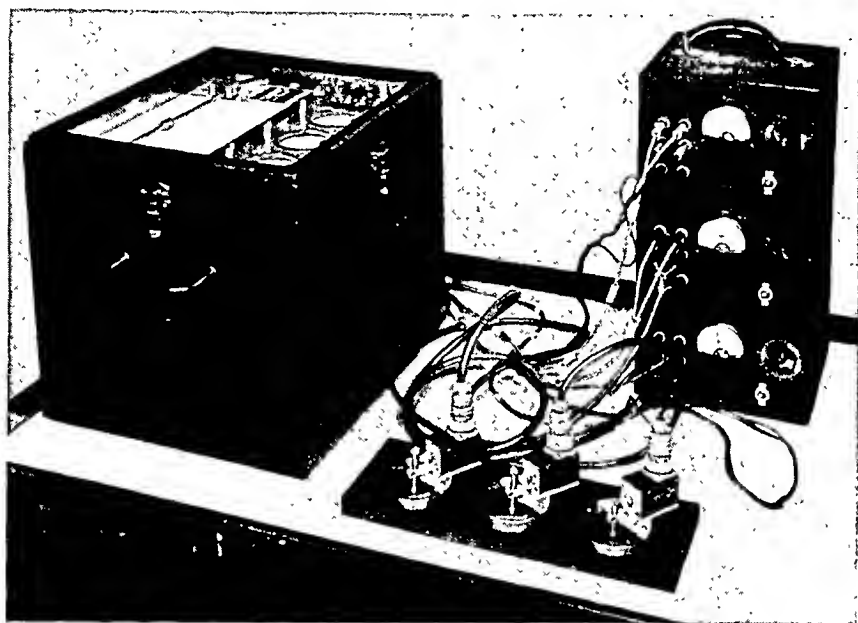


FIG. 1. PHOTOGRAPH OF TOKODYNAMOMETER: THREE STRAIN GAGE PICK-UPS, POWER SUPPLY (RIGHT REAR) AND FRICTIONLESS ELECTROMETRIC WRITING UNIT (LEFT)

3. CONSTRUCTION

The several parts of the TKD now in use by us are as follows: *Strain gage pick-ups*. Satham strain gage dynamometers (Satham Laboratories, Los Angeles), either model YE-4-250 or model G1-4-250, are used. These yield maximum electromotive output with 4 ounces of pressure. The maximum movement of the pin and active element of gage is ± 0.0015 inch. The dynamometer has relatively high frequency characteristics and the output is linear with the stress applied.

The YE model was first used as the only available dynamometer suitable for our purpose. It is the one shown in the illustrations. It has been replaced for several reasons however by the G1 model which

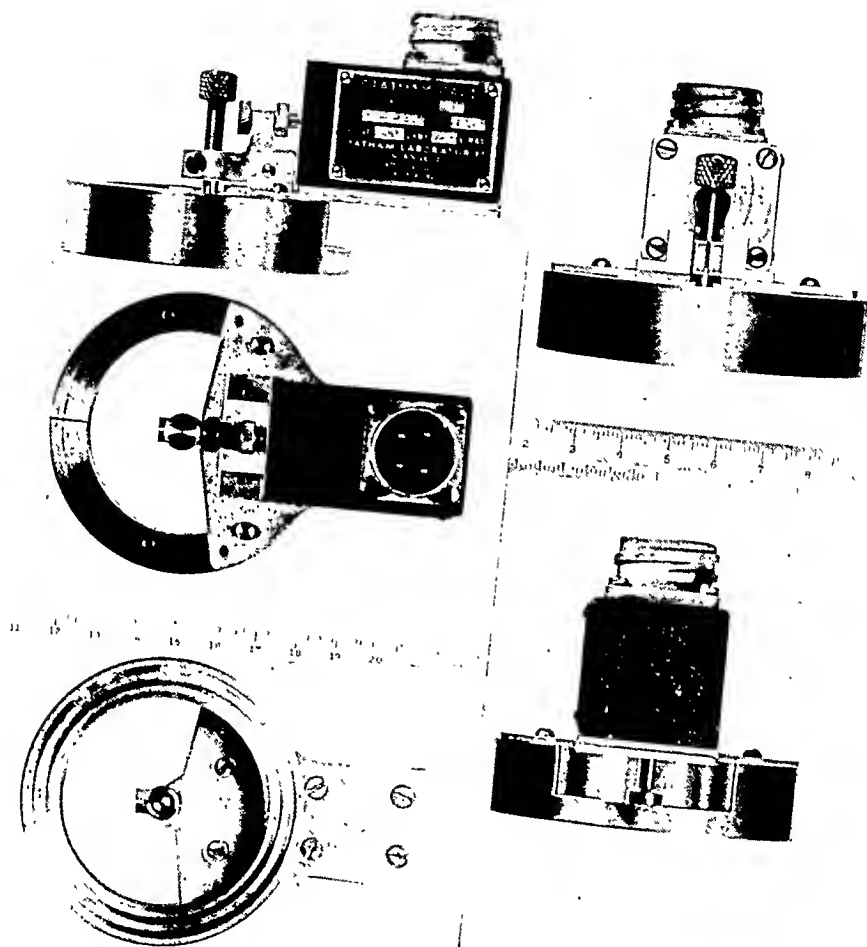


FIG. 2. PHOTOGRAPHS OF THE MODEL YE-4-250 STRAIN GAGE DYNAMOMETER MOUNTED ON THE BRASS RING AND LEVER USED FOR RECORDING UTERINE CONTRACTIONS
a, side view; *b*, top; *c*, bottom; *d*, front; *e*, rear

has identical electrical and tension characteristics. The G1 model is thinner, lighter, and it has no Cannon plug. As a result, it makes a better balanced unit than does the YE model dynamometer. The strain gage is mounted on a heavy brass ring (see figure 2 *a*, *b*,

b, c, d, e) weighing 240 grams. The pin of the strain gage is connected by a simple lever system to an adjustable plunger held vertically, as shown in figures 2 and 3. A stainless steel ball is mounted in the socket at one end of the plunger. Details of the connection between the pin on the strain gage and the lever are shown in the figure 3.²

The essential feature of this unit is that it enables the operator to set the lever at a proper working level without putting a residual strain on the pin, so making it unsuited for use. At the same time,

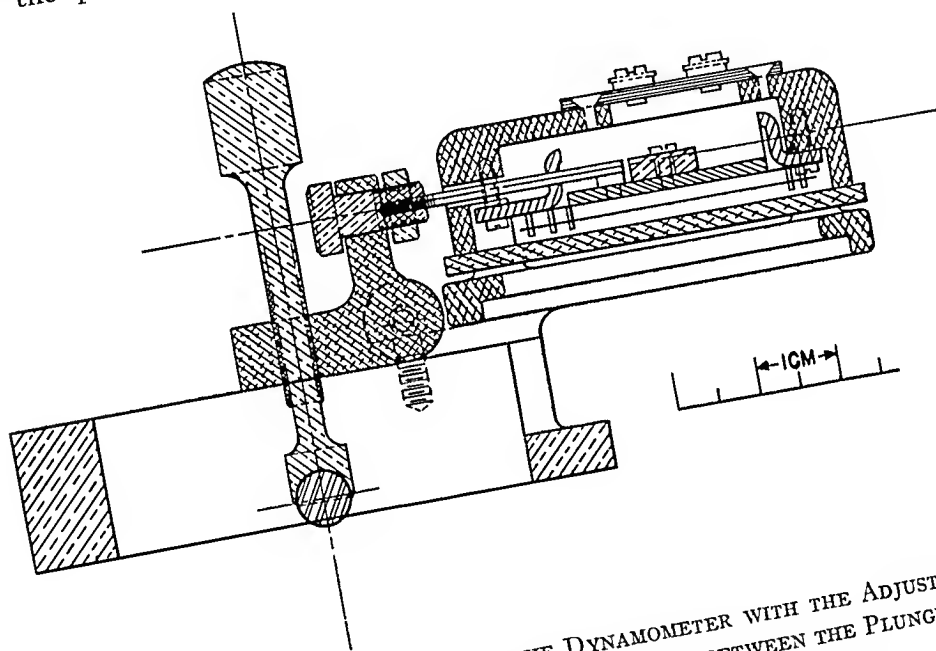


FIG. 3. DETAIL OF INTEGRATION OF THE DYNAMOMETER WITH THE ADJUSTABLE LEVER USED TO RECORD DIFFERENTIAL PRESSURE BETWEEN THE PLUNGER IN THE CENTER AND THE CIRCUMFERENCE OF THE RING

the knurled nut on the screw which binds the lever to the pin can be tightened securely. This holds the lever firmly in place

The development of the pick-up just described was the result of a series of failures. In the first attempt, the gage was mounted vertically on a plate with a brass

² Patterns for the ring, the mounting for the gage and the lever have been made. These units are available through the Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Streets, Baltimore 5, Md. Attention: O. O. Heard.

button on the end of the pin. This button could be adjusted on the abdomen through a hole in the base plate on which the gage was mounted. There were two drawbacks to this arrangement. The unit had such a high center of gravity that it was difficult to secure it on the abdomen, and the uterine contractions tended to work on the pin in a sidewise manner. As a result, uneven records were obtained and eventually the pin was loosened at its soldered joint within the gage. In contrast to this, the lever adopted for use in the pick-up shown in figure 2 serves to eliminate all pressure on the pin except that in an in-and-out direction.

A second method which proved unsatisfactory was that of mounting the gage on its side on a large, light oval aluminum base plate. The dynamometer pin was integrated with the lever now in use. The plate was held in place on the abdomen by strips of adhesive tape. The disadvantage of this was that pressure within the abdomen anywhere along the line of the adhesive tape strips resulted in a change of pressure on the plunger. It was clear from trials with this arrangement that the plunger should touch the abdomen in the center of a circle, and that the area of attachment to the abdomen should be the circumference of the circle. Accordingly, Masonite rings were made with circular cut-outs ranging from two to seven centimeters. By trial and error it was found that rings having a hole 5 centimeters across and a total external diameter of 7 centimeters served best the requirements of sensitivity and compactness. In order to achieve stability, however, the levers and the gages are mounted upon the brass rings shown in figure 2. In this way, the center of gravity is low, and there is sufficient mass to permit development by the uterine contractions of an adequate differential of pressure between the ring and the plunger in its center. A cut-out is made in the brass ring on the side to which the gage is attached to offset the overhanging weight of the gage, as shown in figure 2.

In operation the ring is held in place on the abdomen by application to its underside of a layer of double-coated Scotch tape, as will be described in greater detail below. As a result, only the tape-covered area under the brass ring and the plunger touch the abdomen.

Recorder. The recorder was designed and made to our specifications by the Electro-Physical Laboratories, New York City. This consists of three channels (see figure 4), each of which contains a modified Weston Model 699 microammeter connected directly to the indicating leads from the strain gage. The needles of the galvanometers are extended by fine quartz tubing (just discernible in figure 4 as each extension passes under the black bar in front of the meters) cemented to their tips and projecting through a cut-out in the case. On the tip of each quartz extension a short (2 millimeters) piece of 36 gauge nichrome wire is cemented, like the cross of a T, in a vertical position. This occupies a position between two plates, over the bottom one of

which passes the specially treated heat-sensitive (Permograph) paper shown in figure 4. When the recorder is running, sparks pass from the top plate under the black bar through the nichrome tip to the bottom plate. In passing through the paper, the sparks burn a hole about the size of a pin point. A distributor in the recorder sends sparks

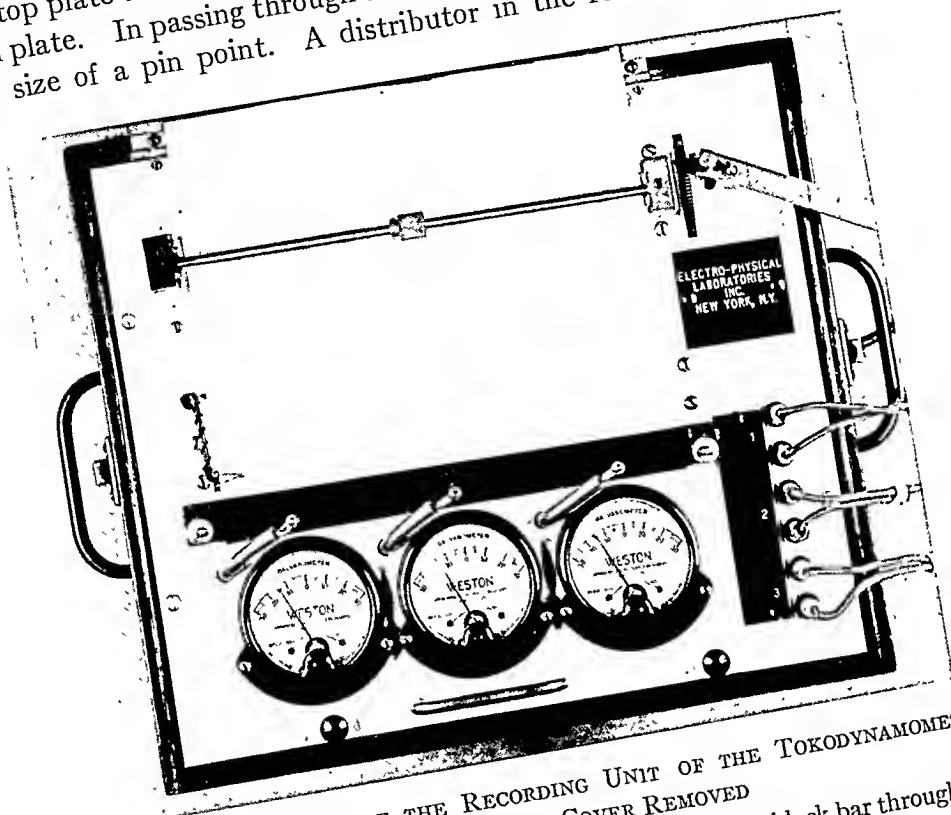


FIG. 4. VIEW OF TOP OF THE RECORDING UNIT OF THE TOKODYNAMOMETER WITH THE PLASTIC COVER REMOVED

Frictionless writing is achieved by sparks passing from the black bar through the heat sensitive paper to the metal plate beneath. The sparks pass through wire tips on the quartz extensions of the microammeter needles. (See these as they pass under the black bar to the left of the spark-distributing wire in each channel.) Two paper speeds are available by means of the lever at the upper right. Time marker is at the left end of the black bar.

through each of the microammeter tips twice a second. Time intervals are marked at the bottom of the paper by means of a heated sapphire point which drops down and touches the paper once every minute. Two paper speeds of 5 and 25 millimeters per minute are available. The tips of the ammeters swing freely without touching

either the metal of the bar above or the paper beneath. The TKD is, therefore, a direct-recording frictionless writing system. The instrument is so sensitive that the top of the recorder is enclosed in a plastic case during operation of the instrument in order to protect it from air currents.

The records of uterine contractility consist of a series of fine holes burned through the paper under each nichrome tip according to its position at the instant of sparking. This position depends upon the amount of pressure applied to the plunger, up to the maximum limit of the gage.

Other types of recorders obviously can be used with the pick-ups which we have devised. For example, it has been ascertained that an ink-writing, two-channel magnetic oscillograph recorder made by the Brush Development Company, Cleveland, works as well as the one described above except that it has one less channel. It consists of one DC amplifier for each channel (number BL 913) and one double channel magnetic oscillograph (number BL 202) modified by adding a slow speed motor (10 r.p.m.).

Power supply and control. Two systems of supplying current input to the strain gages have been used satisfactorily. In both, the input is derived from a 6 volt A battery. In the unit shown in figure 1, the current is controlled by a 200-ohm variable resistance, a 0 to 50 milliammeter and a switch. The output from each gage goes directly to one of the microammeters on the recorder. In the second control circuit, the wiring diagram for a single circuit of which is shown in figure 5, additional features were supplied by the Statham Laboratories. These features simplify the standardization and zero adjustment of the circuit. The zero adjustment potentiometer is used in our TKD to set the needles of the galvanometers at a setting of -14 . In this way, distortion of the record resulting from the radius of curvature of meter needle is minimized. Operation of the standardizing circuit is so designed that it causes a deflection of the microammeter needle equal to one-half the maximum output of the gage. That is to say, a deflection is obtained which is equal to a pressure of two ounces on the pin of the strain gages used in the TKD. By this means the sensitivity of each channel can be adjusted to match the sensitivity of the other two.

The power supply for the Brush Magnetic Oscillograph, when used as part of a TKD, differs from the foregoing in that it does not have a zero adjusting mechanism since this is incorporated in the DC amplifier. Sensitivity control is also available in the amplifier, but the standardizing switch for half the gage output is retained for purposes of electrometric calibration.

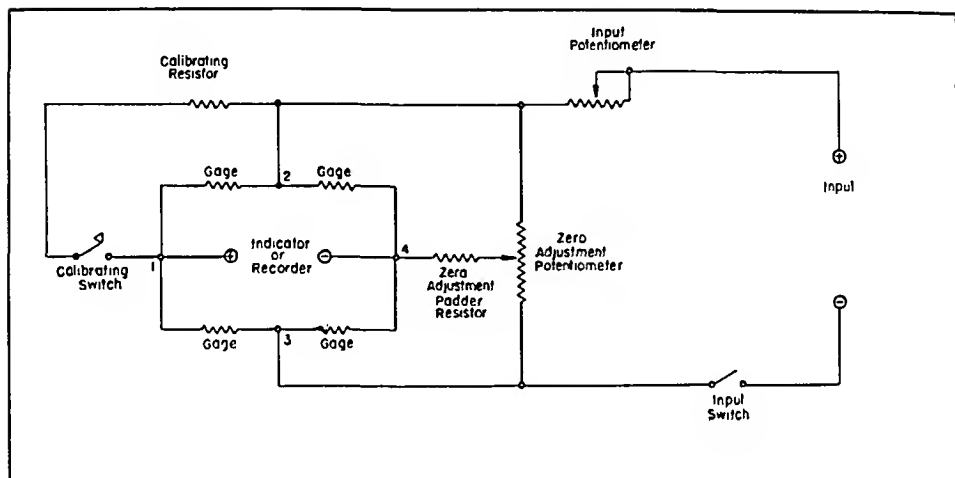


FIG. 5. WIRING DIAGRAM, SCHEMATIC, OF THE CONNECTIONS IN A SINGLE CHANNEL OF THE TOKODYNAMOMETER

Details of the power input control were supplied by the Statham Laboratories.

4. OPERATION

In operation, the tokodynamometer is mounted on a movable table in order that it may be wheeled near the bedside of a patient.

The patients are normally in a supine position for recording, although records have been obtained from patients lying on their sides. In some instances, when vomiting, retching, or straining occurred, the records of uterine activity have been little disturbed, and then only during the period of greatest bodily activity. If records are to be made from the lower uterine segment, the bladder of the patient should be emptied. In very hot weather, it is necessary to wipe perspiration from the abdomen with alcohol immediately prior to application of the pick-ups.

When recording, the gages are secured to the abdomen at the sites of

choice simply by coating the underside of the brass ring of the pick-up with double coated Scotch masking tape, one-half inch wide, as mentioned before. This tape is applied to the ring by taking a six or seven inch length of tape from a dispenser, applying a short length along the under surface of the ring, turning the tape over diagonally to fit the curve and laying down a second short length along the surface. This process is repeated until the entire under surface has been circled once and so is completely coated with tape.

The position the gages will occupy on the abdomen is determined after noting the size, shape, and position of the uterus. A record of this is kept in the following manner: Each pick-up is placed in one of nine standard positions on the abdomen. The distance from the symphysis pubis to the highest point on the fundus is measured and divided into thirds. These three transverse areas are in turn divided into three more by measuring five centimeters on each side of the mid-line of the uterus. Since the brass rings are seven centimeters across, they fit with about one-and-half centimeters on each side when placed in the middle of one of the rectangular areas. The standard areas across the top (fundus) for recording TKG's are designated A, B, and C. Next, across the middle of the uterus, D, E, F and across lower uterine segment, G, H and I. The advantage of employing these fixed areas is that it is possible to classify the data for statistical analysis. In addition, the distance from the top margin of the symphysis pubis to the costal margin directly cephalad is noted. After attachment of the pick-ups, the plungers are adjusted so that the needle of each microammeter is just slightly deflected. At this point, the radius of curvature of each of the needles is recorded by sparking on a stationary paper as the paper-speed switch is set in the neutral position. One or two uterine contractions or manual pressure on the pin of the strain gages will suffice for this. The sensitivity of each channel is fixed to match the others by adjustment of the different input currents in the three channels. With the recorder set to operate at either of the desired paper speeds, turning of a single switch on the recorder starts the paper moving and the sparking mechanism begins to record. Records lasting for periods of time up to four hours and more have been obtained with minimal inconvenience to the patient. During the recording period, the patient may talk, cough, or even change her position somewhat

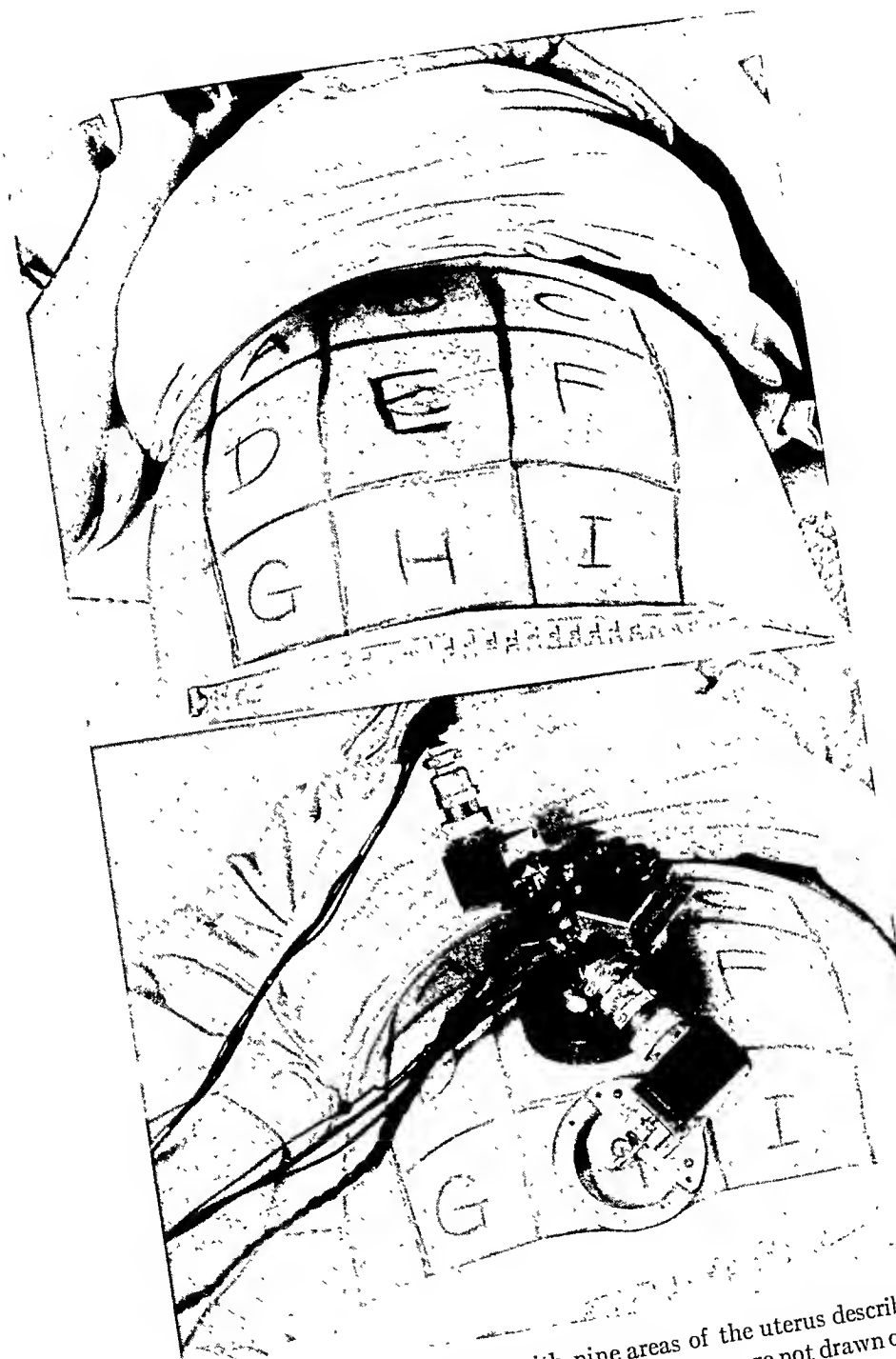


FIG. 6. Top, view of abdomen with nine areas of the uterus described in text indicated by letters A, B, C, etc. In practice the areas are not drawn on the skin, but are determined by the eye.
 Bottom, view of the same area shown above with strain gages in place for recording.

without interference to the record of uterine contractions. Moreover, routine rectal and vaginal examinations may be carried out as well as catheterizations without affecting the record appreciably. The deflections of the galvanometer needles associated with such activities are very transient, or they cause merely a slight shift in the base line. The rhythm of coordinated uterine activity continues without real interruption, and may be both observed or felt by palpation to coincide with the record obtained on the TKD.

TABLE I

List of clinical data included on two hand-stamps which are stamped on each TKD tracing as a matter of record

In addition, an outline of the female torso is stamped on each tracing and the locations of the gages are indicated on this in accordance with the nine areas shown in figure 6.

Name	Admitted
Age	Onset L.
Hist. No.	Membr.
Date	Bleeding
Race	Toxemia
Ht. Wt.	T. P. R.
Parity	Sts. Rh.
L. M. P.	Pelvis
E. D. C.	Pelvimetry
Dur. Wks.	F. H.
Hist. & Rx.	Est. F. Wt.
Exam.	Anes.
Hour	Delivery
Pres. Part.	Duration
Membr.	Child Wt.
Dil.	Add. Notes
Eff.	
Pains.	

The limitations of this method found thus far are that it does not lend itself well to use in very obese patients, and that it does require a limited amount of cooperation on the part of the patient. The latter may be assured in prolonged observations during labor by administering caudal anesthesia.

5. RECORDS

Clinical data. All pertinent clinical data are summarized at the beginning of each tracing. The use of three rubber stamps facilitates

this. A stamp of the torso makes it possible to draw in proper proportion and position the outline of the uterus. The positioning of the pick-ups is similarly marked in the manner described above. The other two stamps permit easy tabulation of all pertinent clinical data as part of the record itself. The abbreviations and symbols used are

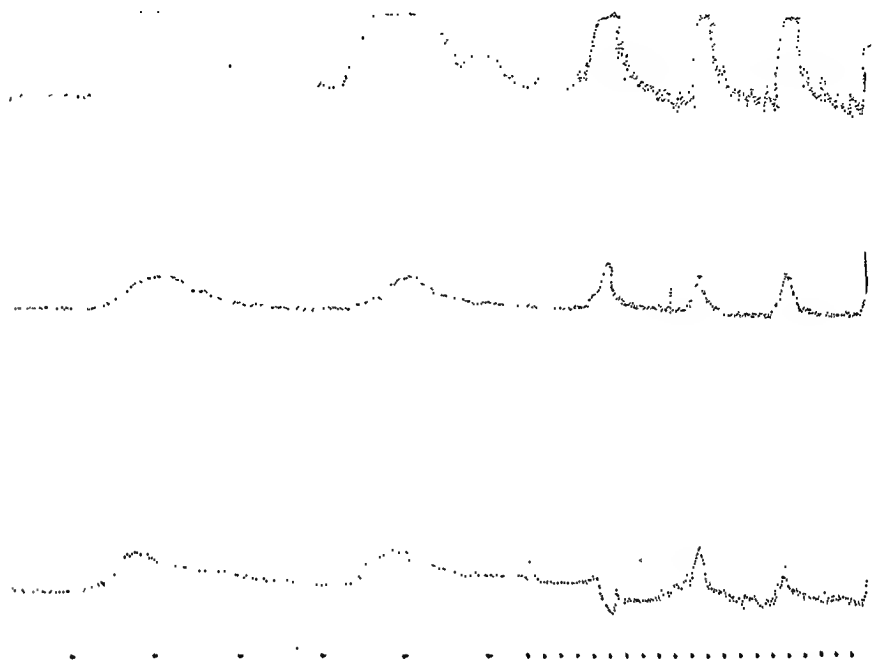


FIG. 7. SAMPLE OF AN UNRETOUCHED TKG, REDUCED ABOUT ONE-HALF

Note the series of fine holes which make up the tracings. Time intervals, 1 minute. The effects of respiratory movements are seen, especially in the upper tracing. Top, fundus recording; middle, over the navel; bottom, lower uterine segment.

the conventional ones employed in the records of the Department of Obstetrics, Johns Hopkins Hospital.

Characteristics of tracings. Examples of sample records are shown in figures 7, 8, 9 and 10. In the first, a section of unretouched record is shown on a slightly reduced scale. The fineness of the holes which make up the three tracings does not permit photographic reduction. When it is necessary to reproduce any length of record photographically the record may be retraced by a ball point pen. This gives a clear

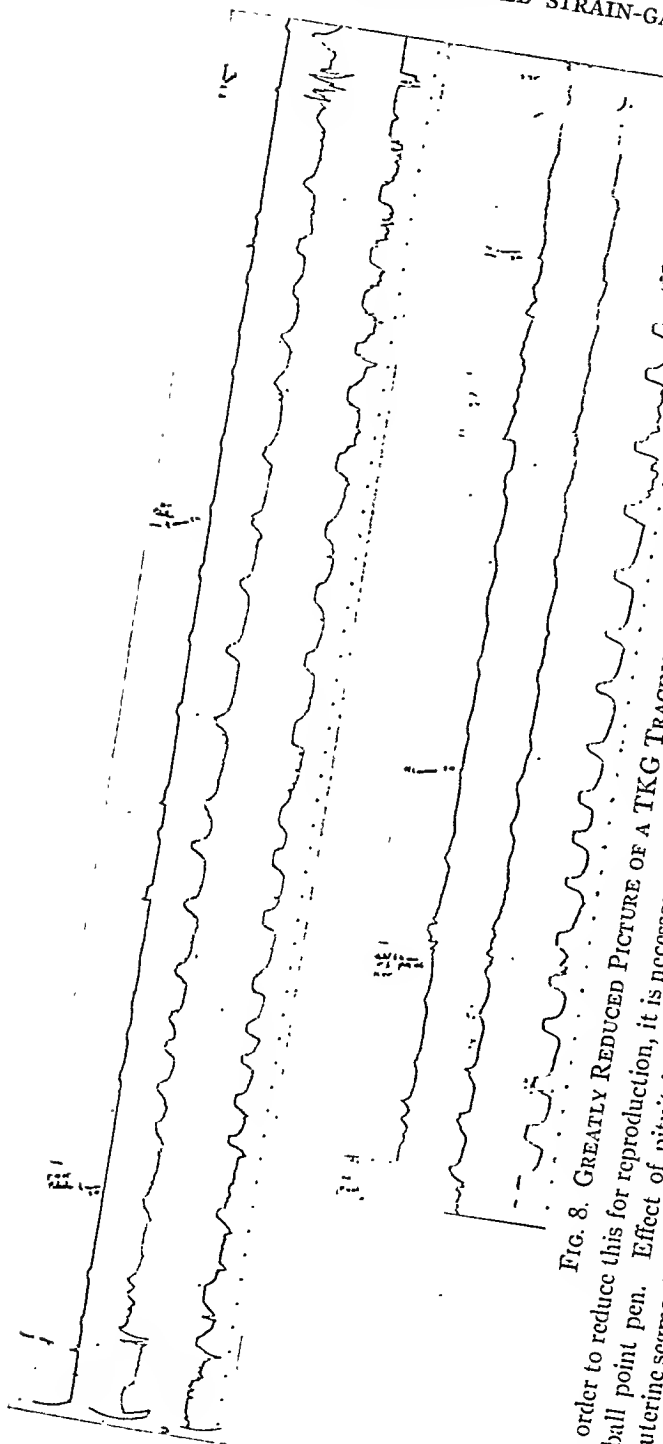


FIG. 8. GREATLY REDUCED PICTURE OF A TKG TRACING FROM FIRST STAGE OF LABOR

In order to reduce this for reproduction, it is necessary to trace over the record with ink. This is conveniently done by means of a ball point pen. Effect of pituitrin, $\frac{1}{4}$ minim intramuscularly, is shown at arrows. Bottom, fundus; middle, at navel; top, minutes of recording have been eliminated. Note the effects of vomiting, just before the break in the record. Record was continuous, but about 40 dilatation passed from 9 to 10 centimeters. Note also the gradual subsidence of the activity in the middle record as cervical Compare with figure 10.

record which will stand very marked reduction, as shown in figures 8, 9 and 10.

Several features of these records require comment. The record, as we have seen above, is made by a series of holes burned by sparks discharged at half second intervals. Because of this the effect of artifacts on the record is interesting. If the fetus kicks or turns, or the

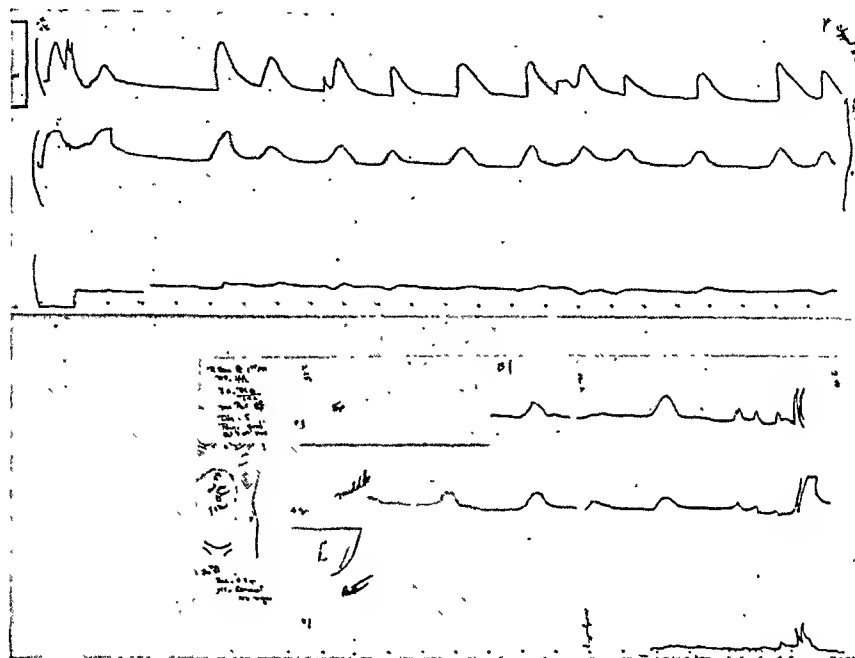


FIG. 9. THE FIRST STAGE OF LABOR AND ONSET OF THE SECOND

The first part of record was from 11:15 to 11:40 a.m. Cervical dilatation, 1 centimeter. At 2:15 p.m. the cervix was dilated 5 centimeters. Pick-ups were placed on same spots as before. With full dilatation at end of record, note onset of reflex abdominal movements in all three tracings.

patient coughs or moves, only one, two, or three single small holes are burned off to one side of the orderly series of holes which record the slower uterine movements. In the records obtained with the TKD described in this paper, it is difficult, therefore, to detect such artifacts. Only when they are sustained for a number of seconds are they easily discerned.

The effects of respiratory movements are likewise of interest. Between uterine contractions, when the indicators are near the base line,

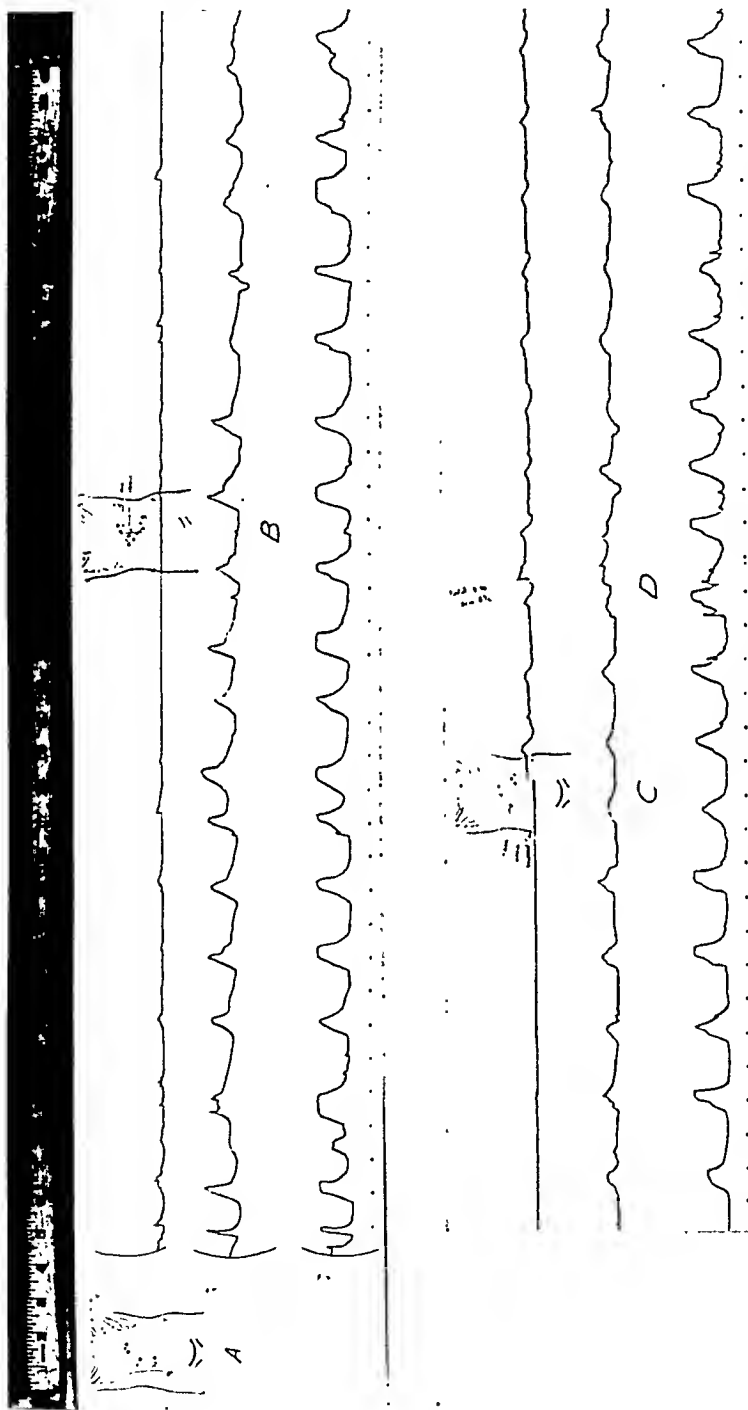


FIG. 10. CHANGE IN ORGANIZATION OF PATTERN OF UTERINE CONTRACTIONS IN THE FIRST STAGE OF LABOR

Record shows activity in the fundus at the bottom; in the middle of the uterus near the navel, and at the top, just below the navel in the lower uterine segment. Except for slight shifts of the last to a position nearer and nearer the middle pick-up, no change in the position of the strain gage was made. Observe the progressive subsidence of the activity at the mid-point of the uterus, so that at the end only the fundus is contracting forcibly. Cervix dilated 1 cm. at start, 2 cm. at end. Record terminated at 12:35 p.m.; spontaneous delivery at 3:09 p.m. Compare with figure 8.

slight movements may be seen occasionally in the channel or channels recording from the fundal portion of the uterus. The effect on the record is to make a line several holes wide as a result of regular oscillations of the microammeter needle which moves through an arc of about one to two millimeters in length. This may be seen in figure 7 in the lead placed over the fundus. During a uterine contraction, the increase in tension on the gage is sufficient to mask completely this slight respiratory effect which is seen when the uterus is relaxed.

Analysis of records. The individual tracing in any one channel is analyzable for the usual time and intensity characteristics of muscle contractions. A specially constructed calibrating board to be described facilitates the analysis of these features. These include, a) frequency of contractions, b) rhythmicity or regularity of contractions, c) relative intensity at the point of recording, d) contraction time, e) total duration of the contractions, f) rates of contraction and relaxation (i.e., slopes of the curves) and g) total relative work per unit period of time. The pattern of activity exhibited by the uterus as a whole may be judged by comparison of the records in the three channels with each other, particularly with respect to their time relations.

The evaluation of the records obtained on the TKD for the above-mentioned points has been solved in the following manner:

a) *Frequency of contractions.* This presents no problem. The number of times the uterus contracts per hour may be measured for records of relatively short duration.

b) *Rhythmicity.* This is determined by measuring the interval of time between the points of onset of successive contractions in a series. The data should be examined, after tabulation, for existence of a trend. If this exists, the fact is noted. If not, and there are sufficient data to warrant it, the data may be treated statistically and analyzed for the standard deviation, standard error of the mean, and so forth.

c) *Total relative work per unit period of time.* This aspect of a tracing may be worked out in the conventional manner, using the planimeter. The sum of the areas between the onset of contraction and the beginning of relaxation under all curves are determined in a given period of time. Since the strain gages are calibrated in grams of force applied to the pin on the gage, any change in the areas under the curves may be determined as a change in work during the contraction phase of a contraction. This may be computed in grams or ergs on an hourly rate or for any other convenient interval of time.

d) *Characteristics of contractions.* The conventional method of assessing the

contraction-characteristics of uterine contractions in a tracing is by simple inspection. Much may be learned by this. For example, a trend in the change in the height (strength) of contractions may be evaluated approximately. The essential thing to remember in this, however, is that the records obtained by the method described in this paper yield limited information: they indicate the change in pressure (i.e., curvature) between the center of the brass rings of the pick-ups and the circumference of the holes in the rings. Consequently, it is difficult to see how any estimate of changes in tonus may be recorded.

A more quantitative way of evaluating the different features of the tracings has been devised. It involves the use of an easily constructed board and a flat piece of Lucite, 6 x 9 inches in width and length respectively. As mentioned before, simultaneous ordinates are laid down in the three channels on the stationary paper. This is to provide a record of the radius of curvature of each of the writing arms and to indicate the position of the time marker at the same time. When the record is ready for study, it is laid flat on the calibrating board with the bottom edge of the paper flush with a raised edge at the bottom of the board. The Lucite sheet is then placed over the paper, its bottom edge held firmly against the raised edge of the board. Lines are drawn with a glass-writing wax pencil over each of the simultaneous ordinates on the tracing. Cross marks are placed on these at 5° intervals for the radius of curvature of each ordinate. This distance has been found in our TKD to be equal to a force of 10 grams applied to the pin of the gage. Either a mark is made over the location of the time marker for the simultaneous ordinates, or the edge of a small cut-out in the Lucite is held at the time mark which is made when the simultaneous ordinates are laid down. It is then possible to analyze certain features of the tracings of the tokodynamographs in the following manner:

(1) *Intensity of contractions.* The point on a particular ordinate at which the uterine contraction commences is noted. The piece of Lucite is then moved to the right until the peak of contraction is reached, the paper and the Lucite being held firmly against the bottom edge of the calibrating board. The distance on the ordinate between the first and the second point shows the force exerted against the pin of the strain gage. As mentioned, this may be read in grams of force.

(2) *Contraction-time.* This may be determined by marking on the time line of the paper the moment of onset of a contraction, using the simultaneous ordinates on the Lucite sheet as a guide and the moment corresponding to the peak of the contraction. The distance between these in minutes or fraction thereof may be estimated with a fair degree of accuracy. For this determination, it has been found that the paper speed at the time of recording should be at least one inch per minute.

(3) *Contraction duration.* This feature may be determined by similar use of the calibrated simultaneous ordinate marker described above. The appropriate distance along the time line from the beginning of contraction to the relaxation phase are noted in the same way that the contraction time is determined. Our

TABLE II

Analysis of the records shown in figure 10

Spontaneous delivery, duration 13 hours. Record begun 6 hours before delivery. Cervix dilated 1 cm. At end of third time period shown in table (after 49 minutes of recording) the cervix was dilated 2 cm. Position of gages: B, mid fundus position; D mid uterus, right of center; H, mid lower uterine segment. See text for system of placing the gages on the abdomen.

CHARACTERISTICS OF CONTRACTION PATTERN										CHARACTERISTICS OF INDIVIDUAL CONTRACTIONS														
Frequency (minutes)		Work ergs				Rate per hour		Per contraction		Gradient conduction time B-D (seconds)		Intensity (grams)			Contraction time (seconds)			Duration of contraction phase (seconds)			Mid uterus: fundus ratio, %		Rate of contraction (grams/second)	
Contraction intervals (minutes)	Average for period	Rate per hour	B	D	H	Fundus	B	D	H	Fundus	Mid uterus	Lower segment	Fundus	Mid uterus	Lower segment	Fundus	Mid uterus	Lower segment	Fundus	Mid uterus	Lower segment	Fundus	Mid uterus	Lower segment
1.2			518	518						45	45	10	20	24	20	38	24	16	2.5	2.1	0.4			
1.8			2,520	260						40	30	8	26	24	20	26	32	0	1.5	1.2	0.4			
2.0			1,080	604						42	38	0	28	20	0	44	20	0	45	1.5	1.9	0		
2.0			650	432						48	35	8	30	24	24	42	33	8	76	1.6	1.4	0.3		
2.0			778	562						42	38	0	32	28	0	42	27	0	64	1.3	1.3	0		
1.2			604	310						38	35	0	35	43	0	55	48	0	87	1.1	0.8	0		
2.3			1,685	216						42	28	0	38	28	0	38	28	0	73	1.1	1.0	0		
1.6			906	390						45	36	0	28	16	0	55	16	0	29	1.6	2.2	0		
2.3			1,290	432						40	30	0	28	24	0	40	24	0	60	1.4	1.2	0		
2.0			1,168	604						48	40	5	36	22	12	56	22	0	40	1.3	1.8	0.4		
2.1			864	216						48	36	3	48	30	12	56	36	20	64	1.0	1.2	0.2		
Aver., 20 min.....	1.8	33	1,100	413						43	35	3	32	26	11	45	28	4	62	1.45	1.46	0.1		

[illegible]

experience to date suggests that the relaxation time is probably of little significance. The reason is that, while the contraction phase is due to displacement caused by the uterine contraction, the relaxation is made up of readjustment of abdominal viscera upon cessation of the uterine contraction. The curve of relaxation is complex, frequently consisting of an initial quick component and of a slow phase of very irregular outline.

(4) *Contraction rates.* The slope of the contraction phase is determinable as follows: the height of the curve, in arbitrary units or in grams, is divided by the contraction-time or the relaxation-time, respectively. In this way, the rate of contraction may be compared during a record and in different parts of the uterus simultaneously. Thus the degree of dissipation of the contraction may be judged very easily in a quantitative manner.

e) *Gradients of activity.* These may be determined very easily and accurately by the use of the Lucite calibrating device described above. The reader may be assured that it is impossible to determine gradients of activity by simple inspection of the record. The procedure we have adopted is as follows:

The moment of onset of contraction in channel #1 is noted on the time abscissa. Similarly, the corresponding contraction in channels #2 and #3 is noted. The differences between these several points indicate the time required for uterine contraction to travel from one gage to the other two, in order. Similarly, gradients of relaxation also may be determined in this way. By appropriately placing the pick-ups on the abdomen and keeping a careful record of the position with the aid of the rubber stamp showing the outline of the torso, described above, it is possible to determine directly, and for the first time, the nature of the pattern of uterine activity in women before and at term and to correlate these positions with the several types of uterine dyskinesias and clinical courses of labor. Our experience to date suggests that the gradient of contraction exists, but that other aspects of the contraction curve of the uterus are more accurately determinable and just as useful.

Examples of each of the above types of analyses are given in table II. These are data taken from a record (figure 10) of normal spontaneous delivery without complications. It is probable that, as additions are made to these data in the future, different values will be obtained since these will be based upon statistical analysis of data from many cases. The present data are offered merely as representative from an individual tracing while the work of gathering numerous data goes on.

DISCUSSION

Several points remain to be discussed in connection with the general aspects of the tokodynamometer and the records which it yields.

In the first place, one may question in what way the records obtained with the TKD differ from those obtained with the Lorand tokograph. One might suspect that, except for the fact that the latter gives but one tracing and the former three, the tracings are essentially similar.

In a fundamental way, however, the two instruments differ markedly from each other. In the TKD, the pick-up shown in figure 2 and described in the text records the force transmitted through the abdomen exerted by the uterus as it changes shape (i.e., the force behind the change of radius of curvature under the brass ring of the pick-up); in the Lorand tokograph the method of applying the unit to the abdomen virtually precludes such localization of effect. It is held in place by means of a strap passed around the body. The instrument can be acted upon, therefore, by any force which tends to alter the length of the circumference of the body under the strap. Local uterine movements do dominate the situation, of course, but it is equally true that remote uterine effects as well as fetal movements, shift in fetal position, or other movement within the area circumscribed by the strap may affect the amount of pressure upon the lever of the tokograph. This, in fact, is what we observed when we first applied our strain gage pick-ups with long strips of adhesive tape. By limiting the adherent surface of the unit to the under side of the brass ring, we procure changes in pressure on the active portion of the gage which are purely local in character.

A second question which may have occurred to the reader, and one which certainly has been of some moment to the authors, pertains to the advantage of, or necessity for, three channels for recording. Would not two channels suffice? It is too early to give a final answer to this question. No doubt for many purposes, especially that of judging the effects of oxytocic and uterine sedative drugs, two channels may serve as well as three. Experience has shown us, however, that there are definite times when three channels yield information not available with two channels. For example, we have seen instances of strong, regular contractions in the uppermost part of the uterus and none in the lower uterine segment in the first stage of labor. This continued throughout the record, but it was associated with strong contractions in the mid-portion which underwent progressive diminution to quiescence as the first stage of labor progressed. Again, we have seen one instance in our limited series of observations, so far, of good dilatation of the cervix in a multiparous woman in which the whole lower half of the uterus was virtually inactive, and the labor was accomplished almost entirely by means of strong, regular contractions in the uppermost portion of

the uterus alone. When we have observed hundreds of cases instead of the five dozen which have been obtained up to the present time, a full, detailed account of the patterns of uterine contractions in different types of labor will be presented. It is evident already, however, that this will be made possible only by means of the three channel TKD now available.

Still another advantage of the three channel unit is that it may well be adapted to somewhat different purposes. For example, it is entirely feasible to connect one channel with a balloon placed in the lower uterine segment during the first stage of labor and employ one of the Statham pressure recording dynamometers (model P8, P6, or P23, with a sensitivity of 2.5 or 6.0 pounds per square inch) for activation of one microammeter. The other two channels may then be used for recording externally the activity of the uterus at two points. In this way, the contractility patterns may be correlated with the force which is dilating the cervix.

In conclusion, a final word may be said concerning the technical aspects of the construction of the TKD. The unit made for us by the Electro-Physical Laboratories serves our present purposes admirably. It has several inherent drawbacks, however: it is relatively large, it is delicate, and the meters are fragile. The records are large, on unlined paper, and are inscribed with a distorting radius of curvature. If a TKD is to be devised for general research, teaching, and clinical uses, it is desirable that it yield records which are rectilinear, on lined paper requiring no photographic procedure, and that the unit be compact and sturdy. The Brush Development unit described in this paper possesses many of these features. It is only a two-channel recorder, however, and there is just as much distortion of the records from the writing levers as there is in our present TKD. It appears that a unit operating on the principle of the heat-writing, rectilinear magnetic oscillograph found in the Sanborn Viso-Cardiette would serve the purpose ideally, except that the unit must be modified to integrate with the direct current Statham gage. It should have slow speed recording and three channels instead of one. It will be worthwhile developing such a unit only if extensive results with the present pilot model indicate that there is a very wide range of usefulness for the TKD in many obstetric clinics.

SUMMARY

1. An instrument has been designed to study the basic patterns of uterine contractility in women before and during labor. In principle it consists of application of strain-gage dynamometers to the abdomen with direct electrometric recording on an electric-spark frictionless recorder. The instrument is called a tokodynamometer or *TKD*.

2. It has an advantage over previous instruments in that it permits study of the origin and spread of uterine contractions. Its construction and operation are not complex. Three Statham strain-gages mounted on brass rings 5 cms. in diameter are secured to the abdomen by double-coated Scotch tape. The plungers are adjusted so that the microammeter needles will deflect with the onset of uterine activity and record by sparks which burn tiny holes in a specially treated heat-sensitive paper.

3. The records (TKG's) obtained can be analyzed for (a) frequency of contractions, (b) rhythmicity or regularity of contractions, (c) total relative work per unit period of time, (d) characteristics of contractions such as (1) intensity of contractions, (2) contraction time, (3) contraction duration and relaxation time, (4) contraction and relaxation rates, and (e) gradients of activity.

4. Because each pick-up records the force behind the change of radius of curvature under the brass rings, this three channel recorder allows a high degree of localization of effect. Hence the spread of the uterine contraction wave or its absence in certain parts of the uterus may be easily demonstrated.

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OXIMETER CONTROL OF ARTERIAL OXYGEN SATURATION IN ANOXEMIA STUDIES¹

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INTRODUCTION

The most widely used clinical method of studying the effects of anoxemia on the electrocardiogram consists of giving the subject 10% oxygen inhalation for twenty minutes and taking records during this period (1a). This method of giving a gas with a constant oxygen concentration is the successor to a previous test in which the subject re-breathed room air to become anoxemic (2a). The re-breathing test was abandoned because it was found that the oxygen concentration of the inhaled gas at the end of the re-breathing period might vary from 13.5% to 6% (2b). The administration of 10% oxygen removed this important variation. This test has been used widely for the detection of coronary disease as determined by the electrocardiographic response and the patient's subjective reaction (1b-g).

Review of the literature reveals, however, that there is a marked physiological variation, as measured by arterial oxygen saturation, among different individuals breathing 10% oxygen and also in the same individual under different circumstances. A recent study of thirty-six normal young men breathing 10% oxygen under basal conditions showed a range of arterial oxygen saturation from 50 to 85% (3). In another instance in which the same gas was used to study coronary function on thirty-seven subjects, the range in arterial oxygen saturation was 52 to 87%, with one subject reaching the low level of 35%

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(1c). Houston recently demonstrated the marked variation of the arterial saturation in the same individual inhaling the same gas, depending on the subject's pattern of breathing (4). Using 10.5% oxygen, he showed a variation as great as 32% in arterial saturation (from 59% to 91%) in the same individual when respiratory minute volume was changed from 8.5 liters per minute to 18 liters per minute. With ordinary respiration this would be comparable to changing the oxygen concentration of the inhaled gas from 16% to less than 8% (3). Changes in the duration of time that a low oxygen gas is inhaled may also cause variations in the arterial oxygen saturation. Subjects placed in a decompression chamber at a simulated altitude of 18,000 feet (approximately equivalent to 10% oxygen at sea level) had an average arterial oxygen saturation of 77% at the end of ten minutes and 71% after fifteen to thirty minutes (5). In summary, then, the present method of using 10% oxygen inhalation for twenty minutes to induce anoxemia may produce an arterial oxygen saturation varying from 50% to 85% in different subjects and in the same subject.

Any procedure capable of producing the same constant level of anoxemia would therefore remove an important obstacle in the standardization and further use of the "anoxemia test" of cardiac function. The purpose of this study is to describe such a procedure. The method consists of giving the subject a mixture of oxygen and nitrogen that will produce a constant level of arterial oxygen saturation as measured by the Millikan automatically-compensated oximeter (6a, b). The oximeter is essentially a small bichromatic photoelectric colorimeter that is attached to the intact ear and measures continuously the arterial oxygen saturation. Its average accuracy, as determined by gas analysis of arterial blood, is (\pm) 3%. It has been used extensively in problems pertaining to aviation (7a, b, c, 5), and to clinical medicine (8a-g, 3, 4, 15, 16).

This preliminary report is concerned with observations on normal subjects at levels of 85%, 80% and 75% arterial oxygen saturation. The lower level was set at 75% for the following reasons: (1) It is the approximate average arterial oxygen saturation resulting from 10% oxygen inhalation (1c, 3, 5); (2) There is considerable cardiovascular stress at this point (3, 9); (3) It provides a safe margin before marked central nervous system symptoms appear (7b).

METHOD

Four normal young men inhaled mixtures of oxygen and nitrogen to induce the desired degree of anoxemia. Each subject was successively studied at levels of 85%, 80% and 75% arterial oxygen saturation on different days.

The oximeter was connected to an ordinary six volt storage battery. The apparatus used to administer low oxygen mixtures was a Heidbrink anesthesia machine (model 302A) in which were placed large tanks of pure oxygen and pure nitrogen. The inhalation tube of the machine was connected to an O.E.M. mask (model 720). The exhalation tube was disconnected so that the subject exhaled into room air. Electrocardiograms were taken with a Cambridge electrocardiograph.

The subject was allowed to rest by lying on the bed approximately one hour. During this time control blood pressure, pulse rate, respiratory rate and electrocardiogram (three limb leads and the precordial lead CF₄) were taken. The oximeter was allowed to "warm up" at least 45 minutes with the earpiece on the standard filter and then on the subject's ear. When the "ear thickness" readings were constant, the "physiological setting" was obtained by giving the subject 100% oxygen.²

The side-arm of the inhalation valve was then attached to the gas machine and the actual test begun. The initial flow rate was approximately 10 liters nitrogen to 2 liters oxygen per minute. The oxygen flow rate or nitrogen flow rate, or both, were then constantly altered to produce the desired level of arterial saturation. Electrocardiograms were taken at the end of 10 and 20 minutes of anoxemia. Ear thickness readings on the oximeter were taken frequently as were blood pressure, pulse and respiration.

² After allowing the oximeter to warm up, the instrumental setting is obtained with the ear piece on the standard filter which simulates the human ear. The ear piece is then screwed to the subject's ear and ear thickness readings taken until constant, indicating maximal vasodilatation. The physiological setting is obtained by turning the "saturation" dial to some known value (98%, if the subject is breathing room air; 100%, if breathing oxygen, or to any other known value, as obtained by arterial blood gas analysis). Continuous readings of the arterial oxygen saturation can then be made. The ear thickness, instrumental setting and physiological settings at the end of an experiment, should check within a few scale units with those obtained at the beginning (6a).

At the end of this period the subject was given pure oxygen until his arterial blood was fully saturated and then allowed to breathe room air. Control electrocardiograms were again taken. At the conclusion of the test, the ear piece was replaced on the standard filter and the instrumental and physiological settings checked.

RESULTS AND OBSERVATIONS

No major difficulty was encountered in producing a given level of arterial saturation. An important factor in attaining this was a smooth induction of anoxemia. This could be accomplished if care was taken to induce anoxemia by decreasing the concentration of oxygen gradually so that the subject was not conscious of any change in inspired gas from room air. To do this, it was found that approximately two minutes were necessary to reach a level of 85% arterial saturation and 3-4 minutes a level of 80% or 75%. It was also found that it was not difficult to maintain a particular level, once reached. It was necessary, however, to make frequent fine adjustments in the nitrogen flow rate throughout the 20 minute period of anoxemia to keep the arterial oxygen saturation within 1 or 2% of the desired level. Occasionally, there were rapid swings in the arterial saturation as great as 5% above or below the desired level. These rapid swings corrected themselves spontaneously within a few seconds without changes in flow rates of oxygen or nitrogen and usually were due to minor disturbances in the room, such as talking or opening of a door.

The ability to produce and maintain any level of arterial oxygen saturation accurately depended more on the subject's being at ease than on any other factor. In one subject (not listed in Table I), who was quite apprehensive, the arterial saturation varied widely from 75% to 90% within a few seconds when 85% was the desired level. The ability to administer the gas mixture with proper dexterity was no great problem and was mastered in one or two trials.

The electrocardiograms were studied for any abnormalities, especially as to changes in the RS-T segments and T waves (Table I). In each of the four subjects studied at the three levels of anoxemia, 85%, 80% and 75%, no striking differences were observed between the various levels. Also, there were no marked differences noted between the electrocardiograms taken after 10 and 20 minutes of anoxemia. In no

TABLE I

SUBJECT	ANOXEMIA		B.P.	ELECTROCARDIOGRAPHIC CHANGES (IN MM., 10 MM. = 1 MV)								OXIMETER READING (SCALE UNITS)		
	Art. ox. sat.	Du- ra- tion		Rate per minute	R-S-T deviation		T wave changes				Ear thick- ness	Physi- ologi- cal setting	In- stru- mental setting	
					I	I-IVF	I	II	III	IVF				
F. J.	%	min.	mm. Hg											
	85	0	132/68	63							75	100	100	
		10	144/70	100	0	1½	-1½	-2	-½	-1½				
		20	132/70	81	0	1½	-2	-2	-½	-2	75	100	99	
	80	0	126/66	83							74½	100	100	
		10	132/64	94	0	1	-1	-1	-½	-2½				
		20	132/68	86	0	1	-1	-½	-½	-2	73	97½	97½	
	75	0	124/62	79							72½	100	100	
		10	148/70	97	0	2	-1	-1½	0	-1½				
		20	134/64	97	0	1	-1	-1	0	0	71	98	98½	
	N. S.	85	0	108/72	63							75	100	100
			10	112/68	79	0	½	0	-½	0	+1½			
		20	114/78	79	0	½	0	0	0	+2	74	99	100	
80		0	104/74	81							75	100	100	
		10	112/72	100	½	½	0	+½	-½	+1				
		20	120/72	100	½	½	0	-½	-½	+2	75	100	99	
75		0	108/62	65							74½	100	100	
		10	112/70	88	½	½	-½	0	0	+½				
		20	108/66	76	0	0	-1	+½	0	+½	73	100	97½	
M. M.		85	0	124/72	79							76	100	100
			10	140/64	100	½	1	-1	-½	0	-1½			
			20	140/70	103	½	1	-1	-½	0	-2	75	100	99
	80	0	112/68	86							78	100	100	
		10	130/72	100	½	1½	-2	-3	+1	-2				
		20	130/68	100	½	1½	-1½	-3	+1	-1	77	101		
	75	0	116/68	75							76	100	100	
		10	140/70	100	0	1½	-1	-2	-1	-2½				
		20		100	0	1½	-½	-2	-½	-2½	75	102	99	
	T. S.	85	0	112/68	73							70	100	100
			10	114/62	81	0	1	+½	-½	-½	-1½			
			20	118/62	71	0	0	0	0	-½	-1	70	99	99½
80		0	112/64	75							70	100	100	
		10	118/70	94	½	2	-1½	-1½	-1½	-1½				
		20	120/64	83	0	1	-½	-2	-1½	-1	68	99	99	
75		0	112/66	68							70	100	100	
		10	130/72	88	0	1	-1½	0	0	-1	70	99	98	

instance was a positive test for coronary disease, as laid down by Patterson, Clark and Levy (1d) obtained. No abnormalities in rhythm or conduction were observed.

The oximeter was found to give the same degree of stability at the different levels (Table I). The final "ear thickness", "instrumental setting" and "physiological setting" did not vary more than 1-2 scale units from the initial readings. It can be said, therefore, that vasomotor changes due to anoxemia (10), and their effect on the ear thickness do not alter the stability of the oximeter in the range of 75-100% arterial saturation.

There was no pain or discomfort experienced from the ear piece. None of the subjects had symptoms at 85% or 80% arterial saturation. At 75% saturation, however, one subject (M. M.) complained of acute frontal headache after 10 minutes and another subject (T. S.) started sweating profusely after 10 minutes anoxemia. Both were promptly relieved when brought up to 80% arterial saturation. No changes in blood pressure, pulse (Table I), or respiration, necessitating termination of a test period, were encountered.

DISCUSSION

The importance of controlling the exact degree of anoxemia in any "anoxemia test" is apparent. The difference in cardiovascular stress at different levels of anoxemia has been demonstrated in normal human beings (3, 7a, c) and animals (11). It is especially important to be able to control the degree of anoxemia in looking for evidence of coronary disease. Variations in arterial saturations in current anoxemia tests may well account for frequent reactions (1a, e, f), "false positives" in normals (1g, 12), and high percentage (approximately 30-50%) of negative tests in persons with presumptive coronary disease (1d, e, f). The possibility of producing an arterial oxygen saturation as low as 50% in a subject with coronary disease, a situation quite possible with 10% oxygen inhalation, may be prevented as the arterial saturation is known instantly at all times during the test.

The method can be applied in anoxemia studies of coronary function under other physiological situations, such as during the administration of carbon dioxide (1c) or drugs (13). It can also be used in studying the effect of different degrees and duration of anoxemia on

other physiological phenomena such as the capillary fragility (8f), or electroencephalographic brain waves (14).

SUMMARY

A procedure for studying the effect of anoxemia on the electrocardiogram has been outlined and its practicability and consistency discussed. Its principle is based on the fact that by continuous fine adjustment of the oxygen concentration in a gas mixture a constant degree of anoxemia, as measured by the oximeter, may be induced in the same and different individuals. It is believed that this method is considerably more accurate than the currently used "induced anoxemia test" in which the subject inhales a gas with fixed oxygen concentration (10%).³

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³ We should like to express our appreciation to Dr. Joseph L. Lilienthal, Jr. for his helpful advice.

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SCOTTISH EXPERIMENTS IN SOCIAL MEDICINE¹

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With each advance in knowledge and experience, the horizon of the art and science of medicine is ever widening. The outlook is no longer limited to the sick-bed or consulting room but ranges over the social, economic, and industrial life of a nation and beyond into the wider expanse of international health problems. More and more it is evident that medical care and the promotion of health in modern civilisation are closely knit with social and economic factors of profound concern to the individual, the family, and the community. The scope of public health has expanded through the impact of the social environment to give rise to the conception of social medicine as a scientific subject which affords a common meeting ground for "preventive" medicine and "curative" medicine—terms which no longer can be definitive of seemingly separate branches of medical practice. War, with its imperative demands on manpower and production, brought home acutely the social and industrial implications of health and sickness. In peace, no less than in war, the relationship between health and working efficiency is of intimate importance to the individual and of prime significance to the national welfare. No nation today can afford to neglect the development of health measures designed to prevent or minimise incapacity resulting from illness or injury.

In the Department of Health for Scotland we have been engaged in several studies and experiments in some difficult and hitherto little-explored fields of social medicine which have opened up several practical questions and point the way to further investigation. Prior to the war, our National Health Insurance Medical Service furnished a vast amount of information capable, after statistical analysis, of giving a measure of the extent to which ill-health and injury rendered people between ages 15-65 years unfit for remunerative employment. The

¹ A De Lamar Lecture delivered at the Johns Hopkins University School of Hygiene and Public Health, October 28, 1947.

amount of incapacitating sickness disclosed became a matter of real concern and a challenge to all concerned with the public health. From 1930 until the outbreak of war, the Department of Health for Scotland published yearly morbidity statistics relating to insured persons. Data were available to show age, sex, occupation, duration of incapacity, and the presumptive diagnosis. Unfortunately, these annual statistics became an early casualty of war when the staff became preoccupied in more serious affairs.

I. A STATISTICAL STUDY OF SICKNESS

For the year ending 30th June, 1938, (the last period for which details are available), the insured population numbered 1.8 million (37 per cent. of the total population). Incapacitating sickness lasting more than three days accounted for the loss of 25.9 million working days. Fresh cases of incapacity arising during the year numbered 417,000. Excluding 44,000 cases of influenza (a disease which can profoundly affect sickness rates), the number of new incapacities was 373,000, or 203 per thousand of the insured population. In addition, there were 31,000 persons unfit for work continuously throughout the whole year, representing 11.3 million lost working days (or 44 per cent. of the total lost time). Many of these patients were no longer suitable for economic employment, and the ailments chiefly responsible were mental and nervous disease, rheumatism, and disease of the cardiovascular and respiratory systems. In a country of about five million inhabitants these figures are of serious import. The returns for eight successive years brought the medical problem gradually into perspective. As might have been expected, sickness rates and duration of incapacity increase with age in men and single women; the rates for married women are dominated by conditions associated with childbirth. What had not been appreciated hitherto was the amount of long-continued or "chronic" incapacity and the fact that between one-fifth and one fourth of chronic cases were under 35 years of age.

With a broad measure of the problem to hand, further analysis was called for as a preliminary to determining what was necessary to minimise the amount of long-continued sickness. From 1937-39 clinical data were obtained for over 50,000 patients in a review of all cases where incapacity lasted for three months or longer. The sample was selective as regards population and sex, and as regards the type of ill-

ness. Obviously fatal diseases were largely excluded from the analysis and, therefore, conditions such as cerebral haemorrhage and malignant disease appeared less frequently than they would in general statistics of morbidity.

Of the first 7,000 patients specially investigated, approximately 53 per cent. were given a prognosis of early recovery; 22 per cent. were liable to recurring incapacity; in 10 per cent. the incapacity would be prolonged but with reasonable prospect of return to some form of employment; 15 per cent. (about 1 in 7) would not likely work again. A sample of 1,000 cases was analysed in some detail. Of these, at the time of examination, 85 per cent. were clearly unfit; about 4 per cent. were able for selected alternative employment. Of the unfit, 45 per cent. were under forty years of age, many of whom were liable to drift into a state of chronic incapacity; 10 per cent. were permanently disabled (1 in 9 of these were under forty years); in a further 11 per cent. the prognosis was doubtful with, at the best, an expectation of only limited or intermittent employment.

Two-thirds of the sample had received hospital treatment either as in-patients or as out-patients. The general medical practitioners reported that lack of facilities hampered diagnosis or treatment in 124 of the 1,000 cases, the chief obstacles being difficulty or delay in admission to hospital or in securing psychological investigation and treatment. Specialist advice was considered necessary and obtained by the Regional Medical Officer of the Department of Health in 89 cases. A new line of approach in treatment was recommended for 350 patients, including hospital admission in 48, psychotherapy in 47, change of work in 29, and return to work for psychological reasons in 16. The final diagnosis of the clinical condition responsible for prolonged incapacity showed that in the sample approximately 1 in 7 were disabled by rheumatism, 1 in 8 by cardiovascular disease (excluding cerebral haemorrhage), 1 in 8 by respiratory disease, and 1 in 10 by peptic ulcer or other digestive disease. Disablement through injury only accounted for less than 8 per cent., fewer than the proportion due to frank psychoneurosis. The cases classified as psychoneurosis did not by any means exhaust the number in which there was a psychoneurotic element. One feature worthy of notice was the tendency in some patients for a sequence of illnesses such as lumbago—influenza—duodenal ulcer.

This preliminary study of the immediate pre-war period, so very

briefly outlined here, furnished objective lessons which led to further experiments during the war, and which serve to direct and guide the post-war development of health measures. In the forefront is the importance of the volume of sickness among the working population and particularly at ages under forty years. Secondly, if we are to minimise the effects of sickness, and if we hope to conserve the working capacity of our wage-earners, the full resources of medical and surgical technique must be brought to bear upon our patients at an early and not at a late stage in illness. Thirdly, where early recovery is not forthcoming, a periodic review of diagnosis and treatment is called for, with close and sufficient cooperation between the personal physician and the specialist services. These formal deductions apart, however, there remains a challenge and a stimulus to a more intensive study of the natural history of disease—not merely its gross pathology but also the early and remediable stages of its evolution, the factors which predispose to it, and the circumstances which initiate it. In the field of industrial accident and injury, some headway has been made in the prevention of mishap and in the recognition of those termed the “accident-prone.” The psychiatrists are adding to the understanding of the psychological factors which produce or complicate ill-health. Little or nothing, however, is known about the conditions which make for susceptibility to cardiovascular “accident,” to respiratory “mishap” or to the “misfortune” of rheumatism. With some precise knowledge of this kind, preventive medicine would truly add to its stature.

II. AN EXPERIMENT IN PREVENTIVE MEDICINE

A further stage in the study of fitness for work was begun early in 1942 in an attempt to minimise breakdown in the health of war-workers. During World War I investigations into industrial accidents and fatigue showed that excessive hours of work lead to lower output and production, with increase in the rates of accident and general sickness. These lessons were seemingly forgotten in the early phase of World War II, although adverse conditions were intensified and increased by the weighty stresses of aerial warfare. As the all-out day-and-night production drive developed and the tension of total war enveloped the civilian population, the prevention of breakdown among essential workers, especially those of younger ages, assumed a new

importance. Young war-workers, and, later, older adults on essential work, whose general health gave cause for concern to their family physician, were given special clinical examination and, where necessary, patients were admitted to war-emergency base hospitals for detailed investigation and to country mansions, functioning as auxiliary hospitals, for intensive convalescence. Within $1\frac{1}{2}$ years some 5,000 patients were dealt with.

Originally those patients were selected who showed symptoms of debility or fatigue, symptoms common alike to physical and mental stress or to early organic disease presenting few diagnostic clinical signs. Later the scheme was extended to include (i) debility after illness where hospital treatment or proper convalescence would expedite recovery of fitness, and (ii) conditions such as dyspepsia, anxiety states, asthma, etc., which are liable to recurrence where stress and strain as etiological factors remain undetected. After a minute clinical survey an assessment was made of etiological and predisposing factors, the immediate nature of the disability, the likelihood of recurrence, and the further measures required.

Of a series of 1,160 patients—23 required routine surgical or medical treatment in ordinary civilian hospitals; 29 had tuberculosis; 79 required change in type or place of occupation; 164 were able to resume their usual employment after advice and re-assurance; 169 were admitted directly to convalescent hospitals; 563 required base hospital investigation.

An outstanding feature was the small proportion of established organic disease. Surprisingly few early organic lesions were missed by ordinary clinical routine methods and required highly specialised hospital investigation. The overwhelming proportion of patients showed vague ill-health, with some degree of debility and often with an anxiety state, due to excessive hours of work, excessive travelling to and from work, and an unsatisfactory dietary. Too often those suffering from physical and mental fatigue carried on to an actual breakdown with, as a result, an unduly prolonged period of incapacity. The great majority responded rapidly to a brief period of rest and change under medical supervision. Recovery was often expedited because the complete medical overhaul made it possible to remove suppressed fears of serious disease. The results indicated that an adequate amount of

rest and recreation is essential to prevent and to combat the fatigue syndrome which, if undetected and untreated, can be the prelude to substantial physical and mental illness. The wise use of leisure as a prophylactic and therapeutic measure of high potency is a subject which deserves a high place in the programme of health education. Our experience also demonstrated (i) the value of careful investigation of the premonitory symptoms and effect of undue fatigue and early debility, and (ii) the need for adequate provision of convalescent hospitals for the management of early departures from health and, indirectly, in relieving the pressure on major hospitals.

III. AN EFFORT TO REDUCE INCAPACITATING SICKNESS BY REHABILITATION

We now had practical experience in reducing health-breakdown to a minimum and preventing the drift into chronic invalidism by prompt and effective treatment. In ordered sequence came the problem of what could be done to restore the sick and injured to the maximum attainable of health, working capacity and social independence. From the early days of the war the Services, particularly the R.A.F., had brought rehabilitation of their casualties to a high degree of efficiency—rediscovering another lesson of World War I which had largely been forgotten or discarded. On the civilian side there had been several orthopaedic out-patient clinics dealing with injured coal-miners, but there was no residential rehabilitation center in Scotland until in January, 1943, the Department of Health for Scotland transferred the large hotel at Gleneagles, Perthshire, from a war-emergency base hospital into a Fitness Centre for disabled miners. Workers other than miners were also admitted later, and in 1947 the unit was transferred to a self-contained wing of another general hospital side by side with a major orthopaedic unit, both units being served by the same orthopaedic team.

We recognised that judicious active rehabilitation would reduce the degree and duration of disability, and that medical responsibility does not end with primary medical or surgical treatment which deals only with a disease process or local injury. The work of the surgeon or physician cannot be considered complete until the patient has attained

the greatest measure possible of physical and mental fitness and has been restored to suitable active usefulness in society.

The ultimate material target of the scheme was the return of men to active employment. It was tempting to limit admission to those patients whose age and disability gave reasonable expectation of a successful issue. This policy, however, would have deprived an important pilot experiment of that harvest of knowledge and experience which is reaped from failure and disappointment. In the early stages, therefore, some patients, medical as well as surgical, were admitted whose prospects were distinctly unfavourable. A wealth of experience soon accumulated from which emerged certain definite principles for the selection of cases and for therapeutic technique. Age was found to be no barrier. Medical cases, no less than the more spectacular surgical cases, can respond to active rehabilitation provided there is no toxæmia or other definite clinical contra-indication.

The programme of treatment was designed not only to restore maximum physical function but also to promote and maintain the patient's will to recover. The patient has to be made to appreciate that while a great deal could be done to him and for him, there is much which he has to do for himself—a principle too often ignored in the past by the medical profession in their management of patients. The keynote, therefore, is activity by the patient, prescribed and controlled by the physician. Passive therapy, such as massage, radiant heat, and manipulation have their uses in the early stages but should merely be means towards the end of active movement, exercise, and recreation. The patient's day from 9.30 a.m. till 4.15 p.m. is fully occupied with the tasks of achieving fitness according to a time table carefully graded on a sound physiological basis. Observing the principle of continuity of supervision and treatment, the surgeon or physician in charge of the patient makes a frequent periodic review of progress, and ultimately a final assessment of fitness for work is made. The assessment of discharge is checked by a follow-up at 2 months and 6 months after the patient has left the hospital. The average stay in the unit ranges approximately from 50 to 80 days.

The results have been encouraging; the clinical assessments, as judged by the 6 months' follow-up reports, have been extremely accu-

rate. Of 1,900 miners and 270 others the results up to June, 1947, have been:—

	MINERS	OTHERS
Fit for former work.....	63%	69%
Fit for light work.....	19%	13%
Fit for alternative employment.....	5%	6%
Unfit at the end of course.....	1%	less than 1%

The remainder consisted chiefly of patients returned to the general hospitals for further primary treatment.

These figures are not statistically comparable with those of the fighting Services. Service casualties were of an optimum age group and usually were in first class condition before injury. The results I have quoted refer to a group of civilians of varying ages up to 70, many of whom had long been in indifferent health.

We have learned that rehabilitation methods cannot cover deficiencies in primary surgical or medical treatment, nor can they be applied *en masse* to patients as a mere supplement to primary treatment. To be of full value, accessory physical and psychological methods must be used at the appropriate moment during and after treatment; sometimes they should begin on the first day in hospital, and sometimes (e.g. prior to meniscectomy) even before operation.

We have learned further that "the patient must be fit if he is to have a job, but, equally, the patient must have a job if he is to remain fit." It has been aptly said that "the final stage in rehabilitation is re-employment, and remunerative work is often the best form of rehabilitation. . . . Much of the time and skill expended on medical care may be in vain if the patient is left indefinitely without work or in employment of a grade lower or higher than that of which he is capable." The physician or surgeon has a responsibility, which cannot be delegated, in determining when his patient is fit for work and for what kind of work he is fit. The medical profession must, therefore, have some appreciation not merely of clinical science but of the conditions under which his patients live and work. There is need for a closer link between the medical services, the social services, and industry if our professional labours are to achieve their time end.

IV. A STATISTICAL INVESTIGATION INTO SOCIAL CONDITIONS

Sociological research is now receiving more attention than hitherto, but we are still woefully deficient in precise knowledge of the influence of social conditions on the health and welfare of the peoples of the world. To build the new world of happiness and prosperity upon the old, we need to study the effect which the changes over the past century have had on the life of the people and ascertain how the people react to conditions of life at the present day.

In Scotland, at the end of the 18th century, and again in the middle of the 19th century, two statistical accounts were made. Sir John Sinclair, who took the first account in the latter years of the 18th century, introduced the phrase into the English language and his interpretation was "an account of the state of the people." These two investigations contain a mass of material about the conditions of life in Scotland 150 and 100 years ago, with information about local customs, local occupations, and local way of life. One of the 160 questions which Sinclair asked related to the most prevalent distempers and their cause. The answers given to this question shed an interesting light on the diseases prevalent in Scotland at the beginning of the last century, their distribution and their relation to local climatic conditions.

Recently, it has been decided to undertake a third statistical account in Scotland and preliminary steps have already been taken in surveying four areas of the country, each under the supervision of a Scottish University. The unit of study is the parish and the following seven headings form the basis of individual accounts:—

1. Physical basis.
2. History of local community.
3. Population.
4. Public and social services.
5. Housing.
6. Agriculture, industries, and commerce.
7. Way of life.

These headings reflect a wide comprehensive undertaking in surveying the whole of the country. The project has been summed up in these words—"The main task will be to describe not merely the physical facts, the public and social services, and the industries of the area, but

to show how, in that environment and with those material conditions, the people live. We shall want to know not only about houses, but about family life; not only about churches, but about what religion means to the people; not only about industries and products, but about the attitude of the people to work and leisure. We shall want to know of any significant changes that have occurred in customs, feelings and attitudes. And we shall want to know their hopes and prospects for the future."

V. MEDICAL CARE IN A LARGE RURAL AREA

Since 1915, there has been, in Scotland, a medical service for the Highlands and Islands which has aroused wide interest and has attracted visitors from many parts to see it in operation. The origin of the Highlands and Islands Medical Service was fundamentally a question of the economics of medical care—a question which today has assumed new importance throughout the civilised world. The problem then, as now, was to apply a remedy without disruption of the established principles of medical practice. Let me give you a brief picture of how in Scotland a State-aided medical service was evolved and developed.

The Highlands and Islands include almost one half of the land surface of Scotland, lying to the North and West, inhabited by some 300,000 people (representing only one-sixteenth or less of the country's population). The region is famed for mountain, glen, moor and sea-board, of unusual beauty and grandeur. Judged by American standards, the scales for distance and population are small, but measured in terms of geographical configuration and of physical exertion and hazard, the provision of medical care in the more remote areas entails effort and endurance of the highest grade. Depopulation over a century has taken its toll of a vigorous and sturdy race; those who remain toil hard to wrest a living from a relatively infertile soil and from stormy seas. Communications even in these days are difficult and journeys are often circuitous and tedious. In the islands there are no railways, and on the mainland the nearest railway station may be several hours distant by automobile. Some of the older inhabitants still living have never seen a railway train.

In 1912 the British Parliament appointed a Committee to examine

the adequacy of the medical services then existing in the region, and to advise "as to the best method of securing a satisfactory medical service therein." The Committee toured the area and obtained first-hand evidence from over 250 witnesses, representing all classes of the community (including over 90 doctors, many of whom had spent a lifetime in the remote areas). Having ascertained the facts and surveyed the problem, the Committee reported that the combination of social, economic, and geographical difficulties in the Highlands and Islands demanded exceptional treatment. The sparseness of population in some parts, the configuration of the country, the climatic conditions, and the straitened circumstances of the people made the medical service uncertain for the people,—onerous and, at times, hazardous for the doctor. In general, medical care was inadequate. The remedy was equally forthright. Medical care and public health services could be developed and correlated provided a suitable grant could be forthcoming from national funds. If doctors could be guaranteed a reasonable minimum income, with adequate nursing and hospital services, well-trained practitioners would be attracted to the Highlands and Islands to combine with a family doctor service such public health duties as would be found useful and expedient. Within eight months Parliament passed legislation establishing the Highlands and Islands Medical Service Fund "for improving medical services, including nursing, in the Highlands and Islands of Scotland; and otherwise providing and improving means for the prevention, treatment and alleviation of illness and suffering therein." Under the Act, schemes were approved covering grants to (i) doctors, (ii) nursing services, (iii) specialist, hospital, and ambulance services, (iv) telegraph and telephone services.

World War I was in progress, however, and although a beginning was made at once, the scheme only got into its stride when hostilities ceased. The results exceeded all expectation. Within a few short years the people of the Highlands and Islands were receiving medical care which became the envy of many in the more prosperous South.

The principles governing the administration of the service have been based upon two primary economic objectives, viz., (i) every person in need of medical attention should be able to secure the services of a fully qualified doctor; (ii) each doctor in the scheme should be assured of a definite minimum nett income.

The few patients able to do so pay the doctor on a fee basis comparable to that obtaining in ordinary private practice. Medical care for those in receipt of public relief is paid for on a block grant basis by the local authority responsible for poor relief. Employed persons contributing to the National Health Insurance scheme are covered by the statutory capitation fee payable for medical benefit. There remain families of the crofter or cottar class, and families of insured workers to whom ordinary medical fees would be an undue burden. For these a schedule of modified fees is laid down which must not be exceeded whatever the distance of the patient from the doctor's residence. Every doctor within the scheme undertakes to observe the underlying principle of the fee schedule.

You will note that as regards private patients and those treated under the statutory services, the Highlands and Islands doctor is exactly in the same position as his brethren elsewhere. The major part of his work, however, is concerned with the families of the crofter-cottar class commonly referred to as "scheme patients." Even were the doctor to receive the maximum fees agreed upon, he would still be far from securing a proper living income. In actual fact, where he applies the *spirit* of his agreement in preference to a strict adherence to the permitted maxima, the doctor invariably adjusts his charges to suit the economic circumstances of his patients—circumstances of which he is uniquely aware by virtue of his position as family friend and adviser, as well as family physician. To supplement his income as compensation for reduced earnings and to offset legitimate professional overheads, each doctor receives a yearly grant from the Highlands and Islands Medical Service Fund. Each individual grant can be varied from time to time according to fluctuations in the circumstances of the individual practice. This year (1947) the target is to provide each doctor with a minimum yearly nett income of £800 after professional overheads have been met. Financial aid has also been forthcoming to provide (i) suitable houses for doctors, (ii) facilities for holiday leave and post-graduate courses at the various medical schools, and (iii) special assistance in the event of serious illness or other emergency.

These arrangements do not make the doctor a salaried servant of the State. In the exercise of his professional duties they leave him as independent and unfettered as his brethren in general practice elsewhere. The contribution of the State is primarily to relieve him from

undue economic burdens and to give him encouragement and scope to practise his profession.

Before the advent of the Highlands and Islands Medical Service, nursing facilities in the Highlands and Islands were haphazard and wholly inadequate. Under the scheme a most efficient nursing service has been rapidly developed. County and district nursing associations were organised and integrated to provide district nurses stationed at strategic points where they could best contribute to medical care. Almost all of these nurses—colloquially and affectionately called “Queen’s Nurses”—are fully qualified in general nursing and obstetrics and, in addition, have had special training for domiciliary work under the auspices of the Queen Victoria Jubilee Institute of District Nursing. The nurses are appointed by the local nursing association but they work directly under and with the local medical practitioners. Words cannot pay adequate tribute to the professional competence and devotion to duty of our nurses in the Highlands and Islands.

Although the nursing service is based upon domiciliary care (including obstetrics and infant welfare), the nurses are actively engaged in the work of the School Medical Service, and in such matters as hygiene and dietetics they bring practical advice to the homes of the people. Their close contact with both doctors and people give them unusual opportunities for the early detection of illness and in effect they function as an efficient health intelligence service.

The local nursing associations are financed by local subscriptions plus grants from the local health authorities supplemented by considerable sums from the Highlands and Islands Medical Service Fund. The financial assistance given has considerably increased the number of nurses employed and ensures that each nurse has a reasonable salary, a suitable residence, means of transport (usually a small car) and a telephone service.

The practical unit in the Highlands and Islands Medical Service is a doctor with one or more fully trained nurses, a closely-knit team designed to bring domiciliary medical care to every individual within a given area. I would draw particular attention, however, to the fact that the doctors and nurses are brought directly into the field of public health. In the isolated areas the doctor enters the service through appointment as a parish Medical Officer for statutory services under the County Medical Officer of Health. He is, therefore, avail-

able to the Medical Officer of Health for immediate action in the locality and he has behind him the Medical Officer of Health for help and guidance in such matters as infectious disease, water supplies, sanitation and emergencies of all kinds. The relationships between Medical Officers of Health and the general medical practitioners is one of cordial friendship and mutual confidence. With the functions of school nurse, health visitor, and social worker centred on the district nurses, and the general practitioners undertaking the duties assigned to them by the Medical Officer of Health, the Highlands and Islands Medical Service has demonstrated how the family medical service and the preventive health service can be integrated to the benefit of the community.

Behind the general practitioner we have placed the resources of a hospital and specialist service. Up to the end of World War I, the existing hospitals were inadequate in accommodation, equipment and personnel, with little possibility of improvement on a satisfactory scale without considerable financial assistance. For obvious reasons first priority was given to a surgical service. This involved the appointment of full-time surgeon-consultants to key hospitals on the Islands and on the mainland, substantial modernisation of layout and equipment, and a considerable increase in nursing staff. Here again the Highlands and Islands Medical Service Fund provided the necessary finance. Substantial grants were made towards the salaries of surgeons and hospital personnel, and towards capital and maintenance expenditure for buildings and equipment.

With its immediate and somewhat spectacular blessings, the surgical service could not fail to stimulate public demand for further extension of specialist services in other fields. Inverness, the capital of the Highlands, was clearly the centre for major development. At Inverness Royal Infirmary, an E.N.T. specialist was established in 1929, and a consulting physician in 1938; in the years between these two appointments suitable grants made possible up-to-date radiological and laboratory facilities. The impact of war in 1939 quickened the tempo of expansion and development. On the outskirts of Inverness, the Department of Health for Scotland built a major base hospital staffed and equipped for major surgery, medicine, orthopaedics, E.N.T., dermatology, gynaecology, and jaw injuries, with a full range of radiological, laboratory, pharmaceutical and physiotherapy facilities. Operationally the War Emergency Hospital was closely linked

with the voluntary hospital, the resources of both institutions being freely interchangeable. To the specialist teams there came two obstetrician-gynaecologists, an orthopaedic surgeon, and a second consulting physician. Ophthalmic and dental services are as yet undeveloped except through the School Medical Service, but the deficiency will be remedied in the forthcoming National Health Service.

The specialists do most of their work in the hospitals to which they are attached. The general practitioners, however, are able to arrange for a specialist consultation at the home of a patient or at some convenient centre such as the house of the local doctor or district nurse.

This brings me to a problem inherent in a medical service in remote areas, viz., communication and transport. Let me illustrate briefly. The configuration of the country, climatic conditions and the inadequacy or, in some parts, the non-existence of roads or paths, often make a trip of some 10-20 miles an arduous or even hazardous undertaking. To reach a patient separated from the nearest road by a few miles of treacherous sea, a doctor or nurse may wait for hours—in winter storms, for days—before the only available craft dare venture on the water. The return journey may have to wait until storm and flood abate. The daily round or a midnight emergency may mean a lonely trip by automobile, boat, pony and a final scramble on foot along a cliff or across a bog. Professional life under these conditions breeds a race of doctor and nurse famed for zeal and daring, wise forethought, incredible patience and marvellous ingenuity!

Some historian may one day record that the automobile above all else revolutionised medical care in the Highlands and Islands. Grants to doctors take into account the condition imposed under the scheme that each practitioner shall provide himself with suitable means of transport. In most areas ambulance services have been provided for the transport of patients to and from hospital, with, in recent years, an increasing use of the air-ambulance. With the cooperation of the Scottish civil aviation authorities, it is now a commonplace event in an acute emergency for a patient in the remote Outer Hebrides to be picked up on the sandy shore by plane, flown South and to be on the operating table in a University hospital within 3-4 hours—a journey which normally would take at least 2 days by mail-boat and rail. The potentialities of the air-ambulance give great promise

for the future, and we await with great interest and expectation the development of the helicopter air-ambulance which promises a solution of a difficult problem facing us in lonely islands and mountainous glens.

The aim of the Highlands and Islands Medical Service has been the provision of medical care within the economic means of all sections of the community, based upon the family doctor with the essential complementary services of nurses, specialists and hospitals. The State contribution has been, first, the necessary finance and, second, a flexible central administration under the Secretary of State for Scotland through the Department of Health for Scotland. The central administration has always been designed to be constructive and not restrictive, intimate and friendly rather than cold and impersonal. Our policy has established and maintained the cooperation and goodwill of all concerned—doctors, local authorities, voluntary agencies, and the general public. Since the conditions in the Highlands and Islands vary so much from place to place, a full appreciation of the particular difficulties of each individual practice is essential for the smooth day-to-day operation of the medical services and for the intelligent planning of expansion and development. Continuous personal professional contact has therefore been maintained between the central Department and the medical personnel in the field through one of the Department's headquarters' medical staff experienced in the problems of rural practice. On this whole-time medical officer devolves the whole range of medical questions arising in the Highlands and Islands area—general and specialist practice, hospitals, nursing, public health and other statutory services. From the outset, his systematic visits to each practice in the area made the medical and nursing personnel aware that an active and helpful interest was being taken in each individual and gave a welcome opportunity for discussion with a medical colleague to those with few opportunities for direct contact with current developments in medical practice and opinion. The best tribute to the administrative handling of the scheme has come recently from the doctors themselves. The Highlands and Islands practitioners, through their British Medical Association Committee, made a special plea to the Secretary of State that under the forthcoming National Health Service, they should continue to serve under the administrative aegis of the Department of Health for Scotland. They based their request upon the fact that

their relationship with the central Department over the past 30 years had been one of confidence and amity.

Standing on the threshold of the forthcoming National Health Service we acknowledge that much remains yet to be done. The people of the Highlands and Islands, however, have enjoyed a measure of medical care with an improved standard of health undreamed of 40 years ago. They are proud of their doctors and nurses, their specialists and their hospitals, and the doctor-patient relationship is the envy of our profession toiling in the cities. We have no difficulty in obtaining doctors, specialists or nurses for any area within the scheme; indeed, the numbers and calibre of applicants for vacancies are frequently embarrassing. The quality of the service is acknowledged to be high. With a satisfactory guaranteed nett income, improved transport facilities, access to specialists, and the means for post-graduate study, the typical Highlands and Islands practitioner is well above the average in professional acumen and knowledge.

I conclude this brief outline by quoting the judgment passed upon the Highlands and Islands Medical Service by one who saw "the former and the latter days"—the late Dr. Eneas MacKenzie of Tain, Ross-shire, a sturdy independent Highlander who was a prince among doctors and a beloved physician to his people. "Considering the geographical character of our country, the Highlands and Islands of Scotland have a medical service unequalled for efficiency in any part of the world. . . . We have lost our sense of loneliness as access to colleagues, specialists and hospitals is easy. This has brought home to the medical practitioner what is the very foundation of his being able to give efficient help to his people, namely, a confidence in his own capacity to deal with any individual case and a knowledge of his own limitations. . . . It is fine to have been allowed to live and work and help into being what has been accomplished."

These, then, are some of the excursions into the wide field of social medicine which we are making in Scotland. They provide no world shattering discoveries but they represent simple efforts to achieve a higher standard of national health. That these endeavours have been possible is a tribute to the happy relationship which exists in our country between all those agencies working towards that objective. I hope they have been of interest to you.

ANTETHORACAL TRANSPLANTATION OF THE STOMACH IN THE TREATMENT OF CONGENITAL ATRESIA OF THE THORACIC ESOPHAGUS

A PRELIMINARY REPORT

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The method of treatment of congenital atresia of the esophagus depends, of course, on the esophageal anomaly encountered. Atresia may result in a complete agenesis or absence of the esophagus, which is quite rare, or consist of a very small defect a centimeter or less in length. The surgical management of the different degrees of atresia requires various methods of construction of the continuity of the gastrointestinal tract. In the event the blind ends of the proximal and distal segments of the esophagus are sufficiently close together, one centimeter or less, primary end to end anastomosis of these segments with closure of the tracheo-esophageal fistula is the operation of choice. But in certain cases, particularly those in which no air is found in the stomach or intestine due to the absence of a tracheo-esophageal fistula or in those patients in which the atresia of the esophagus is so extensive as to make it impossible to bring the blind ends together, it is then incumbent upon the operating surgeon to employ other means to construct an artificial passage between the proximal and distal blind segment.

It is the purpose of this report to call attention to a method of construction of such an artificial passage which is, from a technical standpoint as well as ease and facility of performance, a most gratifying procedure, i.e., the antethoracal subcutaneous implantation of the stomach in infants a few days old in which the diagnosis of extensive atresia of the esophagus has been made. In two such patients complete mobilization of the stomach at the primary operation was accomplished, thus permitting the cardiac end to be brought up to the level of the clavicle followed by an esophago-gastrostomy in a one-stage operation.

Technique of Operation

Under very light drop ether anesthesia, after the usual preparation of the skin, a left subcostal incision 6 cm. in length was made and carried through the underlying fascia. The rectus muscle was divided and the peritoneal cavity opened. The left lobe of the liver was retracted. The stomach and intraperitoneal portion of the esophagus were identified. The atretic portion of the esophagus was divided together with the vagus nerves in the substance of the diaphragm. The left gastric vessels, including the vasa brevia, were ligated and divided. Mobilization of the stomach was completed by dividing the gastrocolic and gastrohepatic omenta, preserving the right gastric and gastro-epiploic vessels. The stomach was withdrawn through the incision and because of the very small size of the infant thorax the cardiac end could be brought up above the clavicle without the slightest tension. An incision 2 cms. in length was made just above and parallel to the clavicle. Between the latter and the original subcostal incision a tunnel was made in the subcutaneous tissue by blunt dissection with a curved clamp. The stomach was then placed under the skin and subcutaneous tissue and drawn up through the tunnel and out through the incision just above the clavicle where the cardiac end was sutured into place. The intraperitoneal portion of the esophagus was brought out through a separate opening in the skin at a slightly lower level and medial to the incision above the clavicle. A tube was inserted in the esophageal opening in order to feed the infant immediately after the operation. This intraperitoneal esophagus was fixed to the adjacent skin with interrupted sutures. The blind end cervical segment of the esophagus was then brought up and into the neck through a separate incision made along the posterior border of the sternomastoid muscle and drawn down to the cardiac end of the stomach by tunnelling under the bridge of skin remaining between the incision above the clavicle and that which was made to mobilize the blind cervical stump. The atretic end was then incised and anastomosed to the cardiac end of the stomach with two layers of interrupted sutures. This anastomosis was then buried by suturing the skin over it.

The postoperative convalescence in both patients was remarkably uneventful except for some leakage of saliva at the point of anastomosis due to the formation of a small fistula. Feedings were made through the tube entering the stomach and even down into the duodenum by way of the opening in the distal esophageal stump.

The two patients were discharged from the hospital but survived only a year when both succumbed to infantile diarrhoea associated with terminal staphylococcus aureus haemolyticus infections beginning in the ears and followed by pyemia with multiple abscesses in the lungs and in one in the liver. The cause of the diarrhoea was not determined. Autopsy revealed that the transplanted stomach in both patients, which had functioned normally as far as the gastric motility and secretion were concerned, had formed a perfectly functioning tube and had remained viable. The small leak in the anastomosis was probably due to slight tension which could have been avoided.

One of the remarkable features was that the infants showed no signs of shock during or following operation probably due to the fact that the blood vessels going to the stomach at this age were so small there was no loss of blood and only an hour's operating time was consumed in each case. The ease of complete mobilization of the stomach in an infant up to ten days of age must be experienced to be fully appreciated.

In spite of the fact that these two infants only survived a year following operation, it is felt that their deaths were due to factors which had nothing to do with the technical side of the operative procedures and that this method of transplantation of the stomach may prove to be a most useful surgical procedure in constructing a new passageway in the alimentary canal in cases of extensive atresia of the thoracic esophagus which do not lend themselves to a primary anastomosis between the two blind segments. The absence of surgical shock in these two cases was most striking and of interest because of the fact that a neurogenic element at this age of five and eight days would probably be lacking.

In his discussion of a paper on congenital atresia with tracheo-esophageal fistula by Singleton and Knight (Trans. Southern Surgical Association, Vol. 55, 1943), Dr. Alfred Blalock of Baltimore, in referring to one of these cases, stated: "Some months ago Doctor Rienhoff had a similar case, and the procedure which was performed differed only in that the vessels at the upper end of the stomach were divided, and this allowed Doctor Rienhoff to place the upper part of the stomach beneath the skin of the anterior wall of the chest."

1. Attention is specifically directed to the fact that the above operative procedure was well borne by two infants five and eight days old which were in a very serious state of malnutrition and in whom there was evidence of a pneumonitis from regurgitation.

2. Although the procedure may sound formidable it may be carried out rapidly and without shock to the patient.

3. The operation of constructing an artificial alimentary canal may be completed in one stage, thus avoiding the multi-stage antethoracic esophagoplasties heretofore employed.

4. Extrathoracic subcutaneous implantation of the stomach avoids, in such poor operative risks, the hazards of intrathoracic procedures.

Brief Abstract of Cases

1) Baby boy Oren, referred by Dr. Schaefer. Mercy Hospital, History No. 49034. Age nine days. Date of birth November 13, 1941. Operated on November 22, 1941; Died November 6, 1942. Baby had normal spontaneous delivery November 13, 1941. Weight 5 pounds 7½ ounces. Did not take feeding well. Regurgitated. Spells of cyanosis. Congenital atresia of the esophagus. Blind pouch opposite the second thoracic vertebra. No gas in intestine in flat plate of abdomen. Patient progressed satisfactorily after operation until August 10, 1942 when he began to lose weight due to symptomatic diarrhea. Developed staphylococcus infection. Weight 9 pounds 6 ounces. Skin soft, velvety.

Physical examination October 12, 1942. Baby is an undernourished male child, apathetic and appears younger than his 10½ months of age. Color is good, no cyanosis or jaundice. There is a scar and small opening on second rib on left side. The bulging mass of stomach can be made out to the left on sternum in the opening below the skin. Several excoriated areas are present over the back and remnants of his previous staphylococcus infection. On October 12, 1942, baby's weight increased steadily for three days and child seemed to be doing very well. Following this the child again developed severe diarrhoea and succumbed November 6, 1942.

2) Baby girl Hart, referred by Dr. T. Terry Burger, Women's Hospital, History No. 22958, five days old, weight 5 pounds 8 ounces. Operation May 13, 1943. Convalescence uneventful. Discharged July 30, 1943. Weight 8 pounds 2 ounces. Fed through tube.

On March 19, 1944. Developed diarrhoea with red throat. Temperature 40. Otitis media. Positive staphylococcus aureus blood culture. Succumbed March 22, 1944, in Johns Hopkins Hospital. History No. H. L. H. A32481.

Personal communication from Dr. Willis J. Potts, Children's Memorial Hospital, Chicago. Infant with congenital atresia of esophagus operated on March 1st, 1948. "Without any difficulty I freed the stomach, brought it up under the skin of the chest and sutured it to the upper end of the esophagus in the neck. There was no shock or loss of blood. Condition good at end of operation. Child died following day, temperature 108°, supposedly an anesthesia death associated with edema of the brain. Circulation of stomach seemed perfect and anastomosis beautifully healed. I was surprised to find with what ease the stomach could be brought up to the collar bone."

PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD MEMORIAL HALL, JANUARY 12, 1948

The Use of Large Skin Flaps for Surface Repair. DR. MILTON EDGERTON.
(Department of Surgery, Johns Hopkins Hospital.)

As a result of the challenge of World War II, new strides have been made in the use of large full-thickness flaps. The success in later surgery to tendons, bones, and nerves depends on effective preliminary surface restoration. The time-honored tube flap is now no longer the method of choice. Particular value has been shown in the use of large "direct" flaps. These may be raised from the abdomen, chest, or thigh, and placed into defects on the lower leg, hand, or arm at the original operation. They may be detached and inserted within a period of about three weeks or even less.

The obvious advantages of these flaps over the use of tubes lies in (1) the great reduction in hospitalization time, (2) the possibility of earlier definitive work on deep structures, and (3) in the actual conservation in flap tissue.

In both military and civilian life, one occasionally meets with a surface defect of such large proportions—particularly on the lower extremity—that the use of a cross-leg direct flap would not offer an adequate donor site. In these cases, the broad expanse of the abdominal and chest skin offers the most logical source of such a flap. It is not technically possible to apply the lower leg beneath such a direct flap. The use of a large tube would involve the loss of many months in transfer, the dangers of a narrow pedicle, and an irreducible waste of tissue from shrinkage.

I would like to mention our experiences here at Hopkins with a new method worked out at Valley Forge General Hospital during the war, under the supervision of Dr. Bradford Cannon. This employs the use of the so-called "jump flap". It is raised with a broad, short pedicle, like similar direct flaps. It differs in being attached first to the arm as a mobile vascular pedicle. The arm carries the flap to its recipient site, for example on the lower extremity. An opposing "mate flap" can be raised on the forearm, at the original operation, and used to cover the raw undersurface of the abdominal apron flap. Split grafts may then be applied to the abdominal wall donor site, and thus a closed wound is secured just as in the use of tubes. The flap is sewed onto the arm primarily with subcuticular sutures. Immobilization of the arm is easily obtained with adhesive strapping. Because of the broad pedicle and consequent good blood supply, the greatest dangers at this stage are from venous thrombosis, stasis, or kinking of the pedicle. A mechanics' waste dressing will combat these effectually.

Either one or two delays may be indicated at the second and third weeks, de-

pending on the size of the flap. It should be pointed out that flaps approaching one square foot in area may be transferred by this means.

Approximately five weeks following the original procedure, and under spinal anesthesia, the flap is then detached completely, the patient flexes at the hip and knee, and the flap may be applied to a defect located at any point on the lower extremity, over the sacrum, hip, or shoulder. It is important that all deep scar be excised and that minute blood supply be obtained. The flap must be placed into the area without tension, allowing approximately ten per cent for linear shrinkage.

Fixation may be obtained with subcuticular sutures of non-absorbable material, and a pressure dressing again applied. It is usually essential to utilize a plaster of paris splint to maintain the flexion of the patient.

At the seventh week, one-half of the attachment from the arm is severed, and after an additional week the arm is separated and its "mate" flap resutured into the forearm. At the same time, the flap is placed into the leg, and the procedure has been completed in seven to nine weeks.

We know of no other method whereby such a large block of tissue could be transferred in a comparable period of time and with comparable safety. I should like to show a few slides of a patient, D. Z., age 17, with traumatic encircling avulsion of the skin from the left lower leg—years of disability, including a draining eczema, and faulty development of the tibia followed. It was necessary for this boy to wear an absorbent dressing at all times. Treatment with a tube flap was first utilized, and after eight months succeeded in covering only a small fraction of the defect. In summary, the use of large full thickness flaps will, in many cases, allow non-union fractures to unite, will permit callus to thicken, and will make it possible to carry out tendon transfers and grafts, nerve sutures, and permit adequate covering for bone grafts. Such procedures, of necessity, require long months of hospitalization. Open direct flaps will give rapid surface repairs, in most instances, with a minimum of heart-breaking complications. Whenever an indirect flap is needed because of the distance of the defect from sufficient donor skin, we feel that the "jump flap" from the abdomen via the arm is the method of choice. It is most useful in thin, limber individuals of a young age group. It requires constant encouragement of the patient's morale. But, under these restrictions, it has proved a versatile and useful procedure, and, in fact, the only solution to a few difficult problems.

Discussion was postponed until all the papers were presented.

Observations on a Skin Homograft After Sixty Days of Pyribenzamine Therapy.

DR. DALE G. FOSTER and DR. EDWARD M. HANRAHAN. (*Department of Surgery, Johns Hopkins Hospital.*)

Homografting (grafting skin from one person to another) is not permanently successful. There is considerable evidence that the reason for this is the develop-

ment in the host of an active systemic immunity to the antigenic foreign tissues of the homograft. Since histamine is frequently released in antigen-antibody reactions involving the skin, it was postulated that possibly histamine was released by antigen-antibody reaction between the homograft and the tissues of the host, and that the histamine so released might play a role in the destruction of the homograft. The authors thus were led to try the clinical effect of an anti-histamine drug, Pyribenzamine, on the survival of a homograft in one case.

A split-thickness graft from a white male was placed in a full thickness skin defect on a colored female. The host received Pyribenzamine therapy for 60 days, at which time the homograft was apparently viable. A biopsy at this time showed living skin with hair follicles and sweat glands, but it could not be determined whether the original graft had survived or whether it had been replaced by tissues from the host. At the end of 90 days the homograft had essentially the same appearance as at 60 days. No conclusions could be drawn from this isolated observation, but it was felt that further investigation was indicated.

Arterial Hemangiomas. DR. WILLIAM P. LONGMIRE, JR. (*Department of Surgery, Johns Hopkins Hospital.*)

In recent years, the term congenital arteriovenous aneurysm has been used for a variety of peripheral vascular lesions. Included in this group is a certain type of lesion which could better be known as arterial hemangioma. These lesions are characterized by (1) a pulsating compressible tumor, (2) enlarged afferent arteries, (3) a systolic thrill and bruit over the tumor, or the afferent arteries, or both, (4) enlarged efferent veins, (5) superficial capillary hemangioma, the so-called port wine stain, in the overlying or surrounding skin, (6) increased local skin temperature. They do not present the true clinical signs of an arteriovenous fistula. Seven such cases have been observed during the past two years. Treatment of these consists of complete local excision of the tumor when possible, or partial excision of the tumor and ligation of afferent arteries when total removal will produce disfiguring or disabling results.

The Treatment of Facial Paralysis. DR. EDWARD M. HANRAHAN. (*Department of Surgery, Johns Hopkins Hospital.*)

The use of fascia to ameliorate the effects of the loss of the seventh nerve is a generally accepted surgical procedure. There is, however, considerable disagreement regarding exactly how the fascial strips should be used to obtain the best result. The principal items of disagreement have to do with points of lateral fixation which might best bring about a balanced face and a certain amount of motion to compensate for the loss of motility in the paralyzed muscles. The best results were obtained by utilization of a combination of procedures which have been tried previously. It is important that the mid points of the lips be solidly immobilized against the pull of the unaffected side. No motion is desirable or necessary at these points which are anchored securely by fascial strips fixed to the temporal fascia. It is desirable, however, that motion be imparted to the line of

expression just lateral to the mouth, and to bring this about one fascial strip is fixed to that part of the temporal muscle which has the greatest excursion. This has given better results than fixation of that strip to other movable points, such as the masseter muscle. Photographs showing the result of this operation will be shown.

Plastic Surgery and Its Relation to Other Specialties. DR. JEROME P. WEBSTER. (*Department of Surgery, Presbyterian Hospital, New York, New York*).

It is difficult to define the field of plastic surgery as it borders on so many of the surgical specialties. A doctor obtaining superior results in the treatment of any type of disorder rapidly accumulates patients suffering from that condition. Therein lie the seeds of specialization. From early Egyptian times to the present, specialization in medicine existed, although usually practice was not limited to one small field. Tagliacozzi, anatomist and surgeon of Bologna (1545-1599), was noted for his rhinoplastic operations and wrote the first book on plastic surgery. When, over two centuries later, Joseph Constantine Carpue in 1816 revived the art of plastic surgery in Europe, certain general surgeons during the nineteenth century performed plastic operations as a part of their surgical practice. Pancoast, Warren, Buck, and Abbe, in this country, became noted for this type of surgery. John Staige Davis was the first to limit his practice to plastic surgery. Blair of St. Louis, originally an anatomist, devoted his time to this field, although he always considered himself a general surgeon.

Certainly a general surgical background is essential for the performance of plastic surgery operations to correct deformities in any part of the body. Halsted, realizing that better work would result if a surgeon's interest were concentrated in one special field, induced men like Cushing, Young, and Crowe, with general surgical backgrounds, to develop neurosurgery, genito-urinary surgery, and otolaryngology. In Halsted's clinic, free skin grafts were extensively used far earlier than in any other clinic in this country. However, he did not support Davis' contention that plastic surgery should be a specialty. His residents received training in a wide field, but the patients sometimes suffered when inexperienced residents, without supervision, operated upon an occasional hare-lip or cleft palate. Proficiency comes with a concentration of thought on the various aspects of any particular medical problem, and a wide experience in treating many cases largely similar in character.

World War I focused the attention of the medical world on the need for specialists in plastic surgery. In 1937, the American Board of Plastic Surgery was formed and became an affiliate of the American Board of Surgery. It was recognized as an independent Board in 1941, with the proviso that its diplomates be trained to cover a wide field of plastic surgery and that at least a two year residency in general surgery after the internship, or its equivalent, precede the two years of training in plastic surgery. Present day general surgical residencies, with rotation through the various surgical services, preliminary to taking up plastic surgery, produce men capable of performing better plastic surgery. However, surgery must pro-

vide places for such training. A man can then determine his natural bent and branch out into plastic surgery if he shows he has a flair for this specialty. An increasing number of residencies in plastic surgery are now available to hasten the acquisition of proficiency in this field, which earlier surgeons had to attain by years of "trial and error" methods.

The plastic surgeon is presented with difficult problems frequently bungled by those in other branches of surgery. It is not requisite that only a plastic surgeon repair deformities in all fields, but surgeons in all fields should be familiar with plastic surgical principles and with what can be accomplished by plastic surgery. The patient will benefit in complicated cases when the plastic surgeon's wider experience and knowledge of what can be accomplished by one of many procedures is utilized. There should be a close union between plastic surgery and the other surgical services. Operations may be performed jointly or with a plastic surgeon in full charge, and with the aid, if necessary, of specialists in the field or fields involved.

What must be sought is the best interest of the patient.

Plastic surgery frequently has to deal with many problems involving the medical as well as all the surgical specialties: metabolism, dermatology, radio-therapy, endocrinology, physical therapy, occupational therapy, pediatrics, neurology, and especially psychiatry. Practical horse-sense in sizing up a patient's mental situation is essential in determining what treatment, if any, should be advised; a schizophrenic, if operated upon, may cause endless worry to or even the death of the plastic surgeon.

Cases are shown which present problems related to a number of medical and surgical specialties; they illustrate deformities covering a wide field which were corrected by plastic surgical methods. "Plastic surgery", as John Staige Davis frequently said, "extends from the top of the head to the sole of the foot."

DISCUSSION

Dr. William P. Longmire, Jr.: Mr. Chairman, I should like to take this opportunity to express our thanks to Dr. Webster for coming here this evening. It has been instructive to see the various cases which he has presented. His discussion emphasizes the wide range of plastic surgery and the correlation of this type of surgery with the other surgical specialties. We have frequently encountered such combined problems at this hospital.

Dr. Foster and Dr. Hanrahan are to be commended for the conservative manner in which their investigation of homografts has been presented. On several occasions in the past, methods have been presented in the literature to insure the permanent survival of homografts, but all such methods have subsequently been proved erroneous.

The information presented, as Dr. Hanrahan and Dr. Foster have emphasized, concerns a single graft; however, their method of investigation is certainly an interesting one which should be explored further.

It seems that the superficial epithelium of this graft has been replaced by the

host epithelium. On the other hand, there are hair follicles and sweat glands deep within the graft which are composed of the original tissue of the donor. As far as I am aware, this is the first time that a similar demonstration has been presented, and it suggests a new avenue of investigation of the homograft problem.

Dr. Edward M. Hanrahan: There is one word I might add to what Dr. Foster has said, and which he did not have time to go into more than briefly. At first glance, it might seem as though the behavior of the pigment in this graft might give a lead as to whether or not it really is a persistence of white color or an ingrowth of colored skin. Unfortunately, pigment behavior about a graft, autograft or homograft, cannot be taken as a measure of that growth. Even the best autografts will sometimes show increase in pigmentation, sometimes show a blanching out to dead white, and a few will stay their normal color. Perhaps you have noticed in the surrounding skin of this colored patient that there was an increase in pigmentation of her own skin in addition to the ingrowth, or in addition to the appearance of pigmentation on the graft. That actually is a very old observation. It was referred to by Reverdin who tried the same thing. Loeb, in 1898, used spotted guinea-pigs. He transplanted black skin onto a white area on the same pig and reversed the process, transplanting white skin onto a black area. The black pigment of the graft would grow onto the white skin surrounding it. Where the white was put in the middle of black skin, the black pigment of the surrounding skin grew into the graft. We have seen on colored patients, where a successful full thickness graft might slough off the superficial layers, that the graft itself will turn white, but pigment returns. The reason for the behavior of these pigmentary changes has never been explained. Loeb advanced several theories. Karg found the same thing in transplants of negroes to whites and whites to negroes, and his were some of the earlier observations on what appeared to be successful homografts. It would help if the pigment would indicate whether or not the graft has persisted, or has been overgrown, but it does not.

Dr. Jerome P. Webster: May I say I think it is splendid to see the amount of interest that is being shown here in plastic surgery. I am delighted.

There was a time when Dr. John Staige Davis had as a patient on Marburg a man of great influence who promised that certain monies would come to the Johns Hopkins Hospital for a surgical clinic and that Dr. Davis would have one floor for plastic surgery cases. Unfortunately, that apportionment was never made in the Halsted Clinic. Dr. Staige Davis was never given the opportunity to develop plastic surgery as he might very well have done, making Hopkins very far ahead in the field at that time. I am delighted to see that this specialty is coming into the foreground and that there is such an interested group of men doing this work.

One should be a little careful in giving priority to anybody on any procedure. While it is excellent to bring out, as Dr. Egerton did, that Dr. Cannon was the first one to popularize that procedure, however I did it fifteen years ago, and I am sure others used the principle longer ago than that; Cannon was not in plastic

surgery at that time. There are so many things in plastic surgery, in any surgery, that have been thought of before, that one has to be very careful in saying who is the originator of a method. J. Mason Warren used a free skin graft long before Reverdin and Thiersch, who are given credit.

Certainly the microscopic section of the skin homograft, which Dr. Hanrahan showed me the latter part of November, indicates that the epithelial covering did come in from the surrounding epithelium. I would not comment regarding the persistence of the original epithelium, but, as he pointed out, certainly the epithelial elements down in the derma did persist and this would indicate that it survived. We know that certain elemental tissues such as the cornea survive as homografts, and that successful corneal transplantation can be performed. That, of course, is the one tissue that we have in the exterior of the body that persists from our fish ancestors. The cornea has to be covered with a moist covering. The tears make it able to live without drying.

About a month ago I saw a homograft in Montevideo in exactly the same position as Dr. Hanrahan's. It had been there approximately the same length of time, and no particular drugs had been given to that patient. Dr. McDonald, one of my former residents, has used more than 250 square inches of skin as a homograft on a burned patient, and this lasted more than ten weeks. I do not mean to say that the graft in this patient is going to come off, and I do not think, from the appearance that I saw in November, that it is. But the graft of the patient in Montevideo looked exactly the same as that of Dr. Hanrahan's, and no drugs had been given. You cannot draw conclusions from *one* case.

I would like to recall the reports of the former observers who said homografts of skin would take, men such as Carrel, who used baby skin and cadaver skin as homografts, and said it survived. Were these men poor observers, or did the homografts actually survive? It is difficult to say. Staige Davis maintained that he knew they survived in at least one or two cases. I wonder if our present difficulty of getting homografts to take, except in identical twins, might not be due to the fact that we are giving a great many protective injections of one thing or another nowadays, such as horse serum, and may be sensitizing ourselves in some way? I don't know. The patient I saw in Montevideo came from the interior of Uruguay where I doubt if any of those injections had been given. It is either a matter of poor observation of the homograft healing in the past, or something has changed the patient's serum so that nowadays we are not getting homograft takes. At least this sensitization is a suggestion to consider.

In regard to the cases reported by Dr. Longmire which he terms arterial hemangiomas, I have observed the same condition many times and also find it is very hard to classify these cases. There was one case I had of hemangioma of the foot, in which a definite bruit was heard in the afferent vessels and yet no definite demonstration could be made of any arterio-venous fistulae by injection of opaque fluid. There was another case with the same condition where there was no bruit, and it was treated by irradiation and the injection of sclerosing fluids.

With the ligation of all the afferent vessels and the injection of sclerosing fluid,

I believe you can get the best result with less danger in those cases where function or appearance might be impaired by operation.

In regard to Dr. Hanrahan's treatment of facial paralysis, he has done some excellent work on the improvement of that deformity. I use, as far as possible, the anterior third of the masseter as a transplant, taking also the periosteum with the muscle to use for suturing because the sutures otherwise will pull out of the muscle fibers.

At a plastic surgery meeting, I saw a surgeon take three portions of the temporal muscle, using local anesthesia, and swing the end of one to the corner of the mouth, one about the eyelids, and one to the frontal muscle. When finished operating, he said to the audience, "You know it is remarkable how fast these patients get these functions back." To the patient he said, "Will you smile?" The patient smiled. "Will you close your eye?" The patient did. "Will you raise your eyebrow?" The patient did. I leave it to you whether that was a successful result or chicanery.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Clinical Examination of the Nervous System, 8th Edition. By G. H. MONRAD-KROHN. Illus. 380 pp. \$4.50. Paul B. Hoeber, Inc., New York, New York, 1947.

This helpful little book on Neurological diagnostic methods is somewhat larger than the previous editions, but follows the same general arrangement. It continues to limit its discussion to the various diagnostic techniques, mentioning specific syndromes only as illustrating the method of examination described. This is excellent for the advanced student with some background in neurology, but is apt to be confusing to the less experienced student, unless used in conjunction with some more complete textbook. The routine methods are well described. There is also considerable discussion of spinal fluid examination, neurological radiology, and angiography.

Several striking omissions are noted. There is no discussion of electroencephalography, which is so widely used as a diagnostic method in this country. There is no mention of the neostigmine test for myasthenia gravis. The chapter on psychosomatic examination is limited to a brief discussion of aphasia. The psychiatric aspects of the examination are brief and reflect purely Kraepelinian concepts, instead of the more modern dynamic approach prevalent in this country.

Despite these limitations, this book will doubtless continue to be popular among students and physicians interested in learning the technique of neurological examination.

G. G. M.

Diabetes and the Diabetic in the Community. BY MARY E. TANGNEY. Illus. 259 pp. \$2.75. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

While primarily written for nurses, this book will be of some value to practicing physicians as well. The routine care of diabetics is thoroughly covered in a clear, concise, easy-to-read style. The author fails, however, to stress the importance of completely drying the alcohol-sterilized insulin syringe before withdrawing protamine insulin.

The short discussions of the physiological disturbances are over simplified and at times superficial, but they serve as an adequate background for the supervision of diabetic patients. The chapter on diabetes and pregnancy treats a very controversial subject in a didactic manner. The parenteral use of massive doses of stilbestrol and progesterone to correct hormonal imbalance during pregnancy is discussed as if it were a routine form of treatment. In reality, it is still in the ex-

perimental stage and too expensive for the average patient. The author states that these hormones must be given by injection because they lose about 90% of their efficiency when given orally. This is not true of stilbestrol, which is highly effective by mouth. Lastly, the author states that diabetes does not develop in the children of diabetic mothers unless the father carries the trait. While this is true, children of diabetic mothers whose husbands do not carry the trait may transmit the disease to their children.

H. F. K., JR.

Diagnosis in Daily Practice. By BENJAMIN V. WHITE and CHARLES F. GESCHICKTER. Illus. 693 pp. \$15.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

The authors have used mortality and morbidity statistics as a basis for their selection of the more common physical and mental disease entities which should be recognized promptly by the practicing physician. Symptoms and clinical findings are then to be evaluated for indications for further diagnostic studies. The proper studies are outlined. A compendium of clinical and diagnostic data and an outline for routine physical examination are given as an aid to enable the physician to recognize and differentiate the diseases.

In a single volume an effort has been made to cover practically the entire field of medicine by means of sign and symptom outlines, abbreviated descriptions of disease complexes, diagrams, and photographs. The attempted scope of the work is of such magnitude that no single subject is in any manner adequately covered.

It is the feeling of this reviewer that good diagnosis cannot be reduced to such simple terms.

C. H. B.

Diseases of Metabolism, 2nd Ed. Edited by GARFIELD G. DUNCAN. Illus. 1045 pp. \$12.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

This volume brings together a group of surveys covering the chief fields of metabolic interest. The contributors are recognized authorities in their fields. The presentations are clear and well organized. The approach is primarily metabolic. A great deal of fundamental information is presented in detail. The vitamins and avitaminoses are described extensively. Endocrinology enters only so far as it affects metabolism. Diabetes mellitus and insipidus, hyperinsulinism, and disorders of the thyroid gland receive individual attention. The anterior pituitary, adrenals, and parathyroids are mentioned under suitable metabolic subjects. The gonads and their metabolic effects receive scant attention. Renal disease and various aberrations of metabolism are well covered.

The editor has brought together a valuable symposium on metabolism and metabolic disorders. Some overlapping and omissions are almost inevitable in a large collaborative enterprise. The description as a "text book for the practitioner" is justified by the large content of clinical interest. Although some chap-

ters might make difficult reading for the practitioner, the usefulness as a reference work would be considerable.

J. A. L., JR.

Nursing. By LULU K. WOLF. Illus. 534 pp. \$3.50. D. Appleton-Century Company, New York, New York, 1947.

Miss Lulu K. Wolf addresses her book on nursing to student nurses in their first year of school. The book is divided into three parts. In Part I, the student is oriented to the profession of nursing, giving her the broad trends and philosophy which have brought nursing to the present day level of an emerging profession. She is acquainted with the nation's health problems and the part played by the nurse in the health programs.

Part II helps the student to become familiar with the hospital as one of the community's health centers. The importance of the patient's introduction to the hospital and its environment is discussed in detail.

Part III orients the student to the numerous practical procedures and principles of nursing involved in patient care. At the end, emphasis is given on the necessity of helping the patient and family plan for the patient's return and care at home.

Miss Wolf's conversational style of writing makes for a clear and personal approach to students. The frequent illustrations help to clarify the details of nursing procedure.

I feel that many instructors of nursing arts will welcome this as a text.

M. F. G.

Nursing in Modern Society. By MARY ELLA CHAYER. 288 pp. \$4.00. G. P. Putnam's Sons, New York, New York, 1947.

"Nursing in Modern Society" is, as its title suggests, a thought-provoking discussion of the problems and opportunities which face the nursing profession today.

The author is a skillful teacher and presents her subject matter in a style which is clear, concise, and altogether delightful. She uses the device of giving just enough about historical events and present issues to make her point, to give the reader perspective, and to whet the appetite for more. This desire for further enlightenment is strengthened by the inclusion of a comprehensive bibliography of source material.

The book is one which will appeal to all persons interested in nursing. Whether the reader is chiefly concerned with legislation, nursing education, public health trends, or how to get more adequate nursing service for the community, he will find these and many more questions offered for thought and study.

The need for a solution of present dilemmas which face the profession is discussed; and original, forward-looking plans proposed. In subject matter of this nature, controversial issues are raised, of necessity, and will arouse wholesome argument.

The message which is emphasized throughout this work is that the professional nurse needs a broader preparation for community and world service, and a new,

sharper awareness of the social forces whose impact is shaping her work. This presupposes drastic changes and a new design for nursing education curricula.

This volume is divided into three sections:

- I The Impact of Social Forces Upon Nursing.
- II The Influence of Social Forces Upon Community Health Needs.
- III Building a Better Future.

It introduces a proposed new series of books with the general title "Modern Nursing." It is the kind of book which should be made available to all nurses in hospitals and public health agencies, and would be invaluable as a text for undergraduate and graduate class discussion groups.

G. L. C.

Surgery of the Ambulatory Patient. 2d Edition. By L. KRAEER FERGUSON. Illus. 932 pp. \$10.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

The new second edition brings the original 1942 volume up to date. This text, since its original publication, has been extremely popular with surgeons, house officers, and students. The reason is not hard to find; the book supplies a real need in dealing constructively with what generally has been known as "minor surgery" from a new and forceful angle. Written by an author and collaborators who have had wide experience in out-patient and office work, the contents are authoritative and practical, though not encyclopedic. The material is well arranged, covering a wide field of congenital, inflammatory, and neoplastic diseases, while a generous section is devoted to the musculoskeletal system, including fractures. A helpful quick reference graphic index of the soft tissues and of the musculoskeletal system is found on the inside cover and flyleaf. There are more than 600 illustrations, many of them demonstrating technics of treatment. The volume is nicely printed on good paper. This book deserves a place in the library or on the desk of any student, surgeon or practitioner who wants an authoritative reference volume for out-patient and office surgical practice.

S. McL.

Textbook of Medicine, 7th Edition. Edited by RUSSELL L. CECIL. Illus. 1730 pp. \$10.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

The seventh edition of this outstanding text follows the general pattern of previous editions. A good deal of new material has been added, and the information and references are uniformly up to date. There are a number of welcome additions to the roster of contributors. The seventh edition is recommended as a remarkably complete and usable text for study or reference.

J. A. L., JR.

BOOKS RECEIVED FOR REVIEW

- Applied Medical Bacteriology.* By MAX S. MARSHALL. 340 pp. \$4.50. *Lea & Febiger, Philadelphia 6, Pennsylvania, 1947.*
- Brain and Intelligence.* By WARD C. HALSTEAD. Illus. 206 pp. \$6.00. *University of Chicago Press, Chicago, Illinois, 1947.*
- Congenital Malformations of the Heart.* By HELEN B. TAUSSIG. Illus. 618 pp. \$10.00. *Commonwealth Fund, New York, New York, 1947.*
- Contemporary American Family, The.* By ERNEST R. GROVES and GLADYS HOAGLAND GROVES. 838 pp. \$4.50. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*
- Emotional Maturity.* By LEON J. SAUL. 338 pp. \$5.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*
- Four Hundred Years of a Doctor's Life.* By GEORGE ROSEN and BEATE CASPARI-ROSEN. 425 pp. \$5.00. *Henry Schuman, New York, New York, 1947.*
- Gynecological and Obstetrical Urology.* By HOUSTON S. EVERETT. 2nd Edition. Illus. 539 pp. \$6.00. *The Williams & Wilkins Company, Baltimore, Maryland, 1947.*
- Jaundice, Its Pathogenesis and Differential Diagnosis.* By ELI RODIN MOVITT. Illus. 261 pp. \$6.50. *Oxford University Press, New York, New York, 1947.*
- Manual of Clinical Therapeutics, A.* 2nd Edition. By WINDSOR C. CUTTING. Illus. 712 pp. \$5.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1948.*
- Manual of Pharmacology, A.* 7th Edition. By TORALD SOLLMAN. 1132 pp. \$11.50. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1948.*
- Minor Surgery.* 6th Edition. By FREDERICK CHRISTOPHER. Illus. 1058 pp. \$12.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1948.*
- Nutrition in Health and Disease.* 10th Edition. By LENNA F. COOPER, EDITH M. BARBER, and HELEN S. MITCHELL. Illus. 729 pp. \$4.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*
- Pathology of Nutritional Disease, The.* By RICHARD H. FOLLIS, JR. Illus. 291 pp. \$6.75. *Charles C. Thomas, Springfield, Illinois, 1947.*
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- Pharmacology and Experimental Therapeutics.* By HAMILTON H. ANDERSON, FUMIKO MURAYAMA, and BENEDICT E. ABREU. 368 pp. \$6.50. *University of California Press, Berkeley, California, 1947.*
- Sexual Behavior in the Human Male.* By ALFRED C. KINSEY, WARDELL B. POMEROY, and CLYDE E. MARTIN. 804 pp. \$6.50. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1948.*
- Surgical Treatment of the Abdomen.* Editors: FREDERIC W. BANCROFT and

PRESTON A. WADE. Illus. 1026 pp. \$18.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*

Textbook of General Surgery. 5th Edition. By WARREN H. COLE and ROBERT ELMAN. Illus. 1160 pp. \$11.00. *D. Appleton-Century Company, Inc., New York, New York, 1947.*

Textbook of Human Physiology. By WILLIAM F. HAMILTON. Illus. 503 pp. \$6.00. *F. A. Davis Company, Philadelphia, Pennsylvania, 1947.*

Textbook of Pathology, A. 5th Edition. By WILLIAM BOYD. Illus. 1049 pp. \$10.00. *Lea & Febiger, Philadelphia, Pennsylvania, 1947.*

TOTAL INTRAVENOUS ALIMENTATION

ITS EFFECT ON MINERAL AND BACTERIAL CONTENT OF FECES

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Certain aspects of total intravenous feeding in the pre- and post-operative management of patients who underwent major operations on the gastrointestinal tract have been previously reported (1). Great reduction in number of stools and amount of feces occurred during total intravenous feeding. At operation the intestinal tract was found empty and in good condition for surgical manipulation.

The present report contains observations on the chemical and bacteriological content of enemas, given at intervals, during total intravenous alimentation, to patients subjected to minor surgical procedures.

METHODS

Diet. The intravenous diet consisted of a solution of casein hydrolysate,² glucose and potassium chloride in water. 10 gm. of nitrogen and 1600 calories per 1.73 square meters of the patient's surface area were given daily (2). A uniform diet of this type was desirable for studies of the vitamin metabolism of these patients, which were carried out in collaboration with Dr. Saul H. Rubin and Dr. Elmer Sevringhaus and are to be reported elsewhere (3). The quantity of Amigen given per 1.73 square meters of surface area contained approximately 33 meq. of chloride, 75 meq. sodium, 330 mg. calcium and 700 mg. phosphorus.

¹ The work described in this paper was carried out under a contract between the Johns Hopkins University and the Office of Naval Research.

² This was usually Amigen which was supplied through the courtesy of Dr. Warren Cox, Jr. of Mead Johnson and Company. In our experience nausea and vomiting have occurred infrequently with this hydrolysate, using the concentrations and rate of infusion as previously outlined (1). In only one of the cases herein reported was vomiting apparently due to Amigen.

3 gm. of potassium chloride were added to the daily fluids of each patient. In some instances sodium chloride was added also. The total volume of fluid per day averaged about 3800 cc. This was infused over an eight hour period, usually from 9 A.M. to 5 P.M. No other water, food or medication was given. The patients were permitted to rinse the mouth with water as desired.

Enemas and Stools. Enemas of sterile physiological saline were administered prior to intravenous feeding and at the ends of various 24-hour periods during the course of the intravenous feeding. In all cases except one, the volume of each enema was 750 cc. The fluid was instilled three times to assure reasonably complete recovery of colonic contents. The volume of the fluid returned was usually greater than 70 per cent of that of the original enema. Smaller returns were often obtained when enemas were given in the first few days following spinal anesthesia, even though a rectal tube was inserted to increase recovery.

In cases in which bacteriologic studies of the feces were made, the first colonic washing was cultured. In some patients a complete collection of feces was made during the 24 hours prior to the start of intravenous feeding. This was diluted to a known volume and cultured as described below.

The initial enema was not analyzed chemically since it did not contain material representing the metabolism of the intravenous feeding period. Subsequent enemas and stools were analyzed.

Chemical. The enema return was mixed thoroughly in a Waring blender and an aliquot taken for determination of nitrogen (4). Another aliquot was ashed and brought into solution for analysis of potassium with the flame photometer as previously described (2), and for analysis of calcium (5) and phosphorus (6).

Bacteriologic. After the return from the enema had been mixed thoroughly in a Waring blender, serial tenfold dilutions were made in beef infusion broth. 1 cc. aliquots of dilutions 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} were poured in rabbit blood agar plates. The plates were incubated aerobically for 24 hours at 37°C . and the number of bacterial colonies counted.

CASE REPORTS

P. I. (J. H. H. #413610), was a 54-year-old man with almost complete esophageal obstruction of nine weeks' duration due to an intrinsic carcinoma. Follow-

ing laparotomy under ether anesthesia, during which metastases to abdominal lymph nodes were found, the patient was given intravenous feeding for six days. Daily 500 cc. enemas were administered. The enema given during the fourth day was not analyzed because of contamination with urine. Bacteriologic studies were not made.

J. A. (J. H. H. #403466), a 17-year-old boy, was admitted for right inguinal herniorrhaphy. He was fed wholly by vein for seven days. Herniorrhaphy was performed under spinal anesthesia on the third day. Daily enemas were administered.

H. A. (J. H. H. #403957), was a 37-year-old man with an umbilical hernia. Herniorrhaphy was carried out under spinal anesthesia on the third day of intravenous feeding, which was given for nine days. Daily enemas were administered. The enema fluid recovered at the end of the third day was clear and scanty. Through error it was discarded. Bacteriological studies were not made.

G. R. (J. H. H. #410439), was a 28-year-old man with a left inguinal hernia. Herniorrhaphy was performed under spinal anesthesia on the second day of intravenous feeding, which was given for seven days. The first day, while receiving a casein hydrolysate other than Amigen, the patient experienced severe nausea and vomiting which were followed by several spontaneous stools. The following days Amigen was given without reaction. Enemas were given at the end of the first and seventh days.

B. A. (J. H. H. #318701), a 16-year-old boy, was admitted for repair of an umbilical hernia. Intravenous feeding was given for eight days. Herniorrhaphy was carried out under spinal anesthesia on the third day. An enema was given at the end of the eighth day.

O. F. (J. H. H. #413610), was a 42-year-old man with an epigastric hernia. A complete collection of feces was made in the 24 hours prior to the start of intravenous feeding. Herniorrhaphy under spinal anesthesia was performed on the third day of intravenous feeding which was given for five days. Vomiting occurred several times during the last two days of intravenous feeding, but there were no spontaneous stools. An enema was administered at the end of the fifth day.

J. O. (J. H. H. #197187), a 33-year-old man, was admitted for excision of an ischiorectal sinus tract. A complete collection of feces was made during the 24 hours prior to the start of intravenous feeding. A spontaneous stool occurred during the first day of parenteral feeding. Operation was performed under spinal anesthesia on the fourth of seven days of intravenous feeding. A casein hydrolysate other than Amigen was given, and vomiting of small quantities of material occurred two to six times per day during the last three days. There were two spontaneous stools during the last day of intravenous feeding. Enemas were given at the end of the third and seventh days.

T. H. (J. H. H. #305627), was a 56-year-old man with pruritis ani. A complete collection of feces was made during the 24 hours prior to intravenous feeding. Alcohol injection was carried out under spinal anesthesia on the sixth of seven days of intravenous feeding. There was a small loss of fecal material at operation. Enemas were given at the end of the fifth and seventh days of intravenous feeding.

TABLE I

Recovery of minerals and nitrogen from colon by enemas and stools during total intravenous feeding

PATIENT	DAY	SPECIMEN	VOLUME OF ENEMA RECOVERED	CALCIUM	PHOSPHORUS	POTASSIUM	NITROGEN
Group 1							
B. A.	8	Enema	per cent 79	mg. 7	mg. 6	meq. 1	gm. 0.1
		Amount per day		0.9	0.8	0.1	0.01
F. R.	2	Stool	48	664	336	18	0.1
	8	Stool		319	332	16	0.3
	8	Enema		11	15	1	0.1
		Total		994	683	35	0.5
		Average per day		124	85	4	0.1
H. I.	7	Enema	93	6	3	1	0.0
		Amount per day		0.9	0.4	0.1	0.0
Group 2							
P. I.	1	Enema	79	4	1	0	0.0
	2	Enema	60	5	1	0	0.0
	3	Enema	100	19	8	2	0.8
	4	Enema	Not analyzed				
	5	Enema	60	28	17	1	0.0
	6	Enema	76	137	35	1	0.1
		Total		193	62	4	0.9
		Average per day		39	12	0.8	0.2
J. A.	1	Enema	67	166	32	0	0.3
	2	Enema	80	8	6	1	0.6
	3	Enema	33	5	1	1	0.3
	4	Enema	33	2	1	0	0.1
	5	Enema	70	4	2	1	0.2
	6	Enema	73	22	2	2	0.2
	7	Enema	93	77	41	8	0.7
		Total		284	85	13	2.4
		Average per day		41	12	2	0.3

TABLE 1—*Concluded*

PATIENT	DAY	SPECIMEN	VOLUME OF ENEMA RECOV- ERED	CALCIUM	PHOS- PHORUS	POTAS- SIUM	NITROGEN
			<i>per cent</i>	<i>mg.</i>	<i>mg.</i>	<i>meq.</i>	<i>gm.</i>
H. A.	1	Enema	70	111	96	6	0.1
	2	Enema	73	26	28	4	0.8
	3	Enema	Not analyzed				
	4	Enema	60	4	2	0	0.0
	5	Enema	89	6	13	1	0.5
	6	Enema	84	9	31	7	1.4
	7	Enema	71	259	240	8	1.1
	8	Enema	81	74	30	9	1.8
	9	Enema	89	231	119	10	0.5
		Total		720	559	45	6.2
		Average per day		80	62	5	0.7
Group 3							
O. F.	5	Enema	87	139	32	6	0.8
		Amount per day		28	6	1	0.2
G. R.	1	Stool		1,670	985	25	0.3
	1	Enema	88	23	33	2	0.7
	7	Enema	85	15	30	5	1.0
		Total		1,708	1,048	32	2.0
		Average per day		244	150	5	0.3
J. O.	1	Stool		470	356	11	0.5
	3	Enema	93	6	3	0	0.0
	7	Stool		513	344	18	1.2
	7	Enema	96	4	3	0	0.0
		Total		993	706	29	1.7
		Average per day		142	101	4	0.2

Because of the loss of feces at operation, excretion studies on this patient are not included.

F. R. (J. H. H. #310860), a 56-year-old man, was admitted for repair of a right inguinal hernia. There was no anemia or leukocytosis, but he was found to have eosinophilia of 20 and 11 per cent by two determinations. Two specimens of feces were examined by a parasitologist who found no parasites. Feces were collected

during the 24 hours prior to eight days of intravenous feeding. Herniorrhaphy under anesthesia with sodium pentothal and ether was carried out on the fourth day. The patient had spontaneous stools on the second and eighth days. An enema was given at the end of the eighth day.

H. I. (J. H. H. #422744), was a 57-year-old man with bilateral inguinal hernias. Herniorrhaphy was performed under anesthesia with sodium pentothal and ether on the third of seven days of intravenous alimentation. There were no spontaneous stools. An enema was given at the end of the last day of intravenous feeding.

RESULTS

Chemical Studies. The data, obtained by analyses of enemas from each patient, are given in the three subdivisions of Table I. Patients who received infrequent enemas are in group I; those who received daily enemas are in group II; patients who experienced nausea or vomiting during the intravenous alimentation are listed in group III.

There are three patients in group I. Each of these patients received an enema on the morning that intravenous feeding began, and no subsequent enema was given until the course of intravenous feeding was completed. Patient B. A. had no spontaneous evacuation. The enema given at the end of eight days of intravenous feeding recovered 7 mg. of calcium, 6 mg. of phosphorus, 1 meq. of potassium and 0.1 gm. of nitrogen. H. I. had no spontaneous evacuations. An enema given at the end of seven days of intravenous feeding recovered 6 mg. of calcium, 3 mg. of phosphorus, 1 meq. of potassium and 0.0 gm. of nitrogen. F. R. had two spontaneous evacuations (one on the second day of intravenous feeding and one on the last day. An enema was given on the last day after the spontaneous stool). Total recovery of material from the colon over the eight day period of intravenous feeding was 994 mg. of calcium, 683 gm. of phosphorus, 35 meq. of potassium and 0.5 gm. of nitrogen.

Group II. Three patients received daily enemas. 193 mg. of calcium, 62 mg. of phosphorus, 4 meq. of potassium and 0.9 gm. of nitrogen were recovered from P. I. in six days; 284 mg. of calcium, 85 mg. of phosphorus, 13 meq. of potassium and 2.4 gm. of nitrogen from J. A. in seven days; and 720 mg. of calcium, 559 mg. of phosphorus, 45 meq. of potassium and 6.2 gm. of nitrogen from H. A. in nine days.

Group III. Three patients experienced nausea and vomiting. O. F. received no enemas in five days of intravenous feeding during the

TABLE II

Recovery of viable aerobic bacteria from the colon by enemas and spontaneous stools during total intravenous feeding

Day 0 is the day of oral feeding prior to the start of intravenous feeding.

PATIENT	DAY	MATERIAL	ENEMA RECOVERED	VOLUME DILUTION OF STOOL	BACTERIAL CONCENTRATION PER CC. $\times 10^8$	TOTAL BACTERIA RECOVERED $\times 10^9$	NET CHANGE IN ENEMA COUNTS (APPROXIMATE)	N, GRAMS TOTAL
			<i>per cent</i>					
F. R.	0	Stool		200	5	1.0		0.10
	0	Enema	91		8	5.4		
	8	Enema	47		48	16.9	3-fold incr.	0.10
T. H.	0	Stool		200	6	1.2		
	0	Enema	61		1	0.5		
	5	Enema	52		3	1.2		
	7	Enema	93		3	2.1	4-fold incr.	
B. A.	0	Enema	96		0.3	0.2		0.10
	8	Enema	79		25.3	15.0	75-fold incr.	0.01
O. F.	0	Stool		270	6	1.6		
	0	Enema	107		1	0.8		
	5	Enema	87		158	103.0	128-fold incr.	0.80
J. A.	0	Enema	55		2	0.8		0.30
	2	Enema	80		19	11.4		0.60
	3	Enema	33		15	37.1		0.30
	4	Enema	33		29	71.6		0.10
	5	Enema	70		21	110.0		0.20
	6	Enema	73		73	399.0		0.20
	7	Enema	93		105	734.4	917-fold incr.	0.70
J. O.	0	Stool		200	11	2.2		0.50
	0	Enema	100		9	6.7		
	3	Enema	93		3	2.1		0.00
	7	Enema	96		9	6.5	No change	0.00
G. R.	0	Enema	88		0.04	0.026		0.30
	1	Enema	88		0.02	0.013		0.70
	7	Enema	85		0.02	0.013	2-fold decr.	1.00
H. I.	0	Enema	136		26	26.4		0.00
	7	Enema	93		1	0.7	37-fold decr.	0.00

last two of which nausea and vomiting occurred. An enema at the end of this time recovered 139 mg. of calcium, 32 mg. of phosphorus, 6 meq. of potassium and 0.8 gm. of nitrogen. G. R. had a spontaneous stool during the first day of intravenous feeding, during which nausea and vomiting occurred. 1708 mg. of calcium, 1048 mg. of phosphorus, 32 meq. of potassium and 2.0 gm. of nitrogen were recovered in one stool and two enemas during the seven days of intravenous feeding.

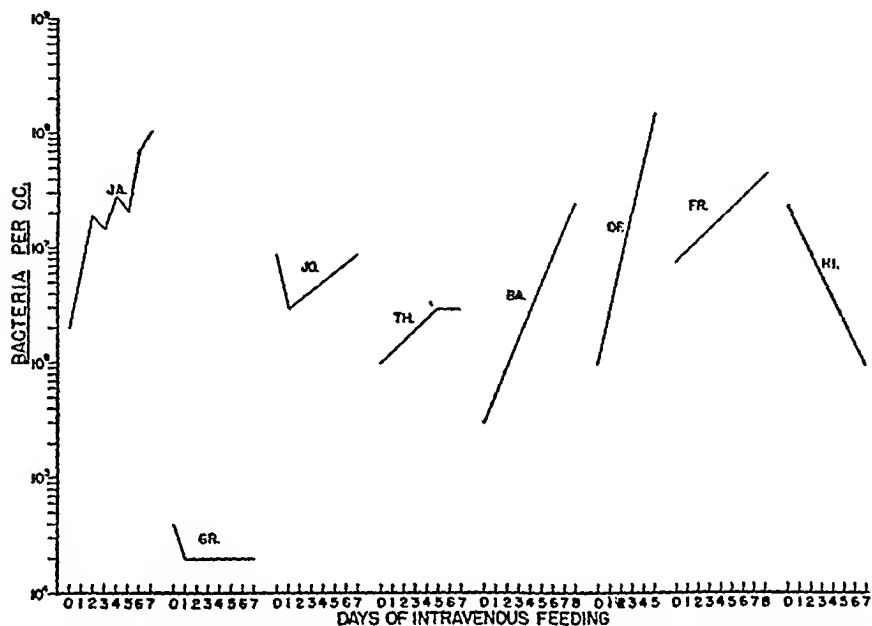


CHART I. RECOVERY OF VIABLE BACTERIA FROM THE COLON BY ENEMAS DURING TOTAL INTRAVENOUS FEEDING

The concentrations of bacteria in returned enema fluids are plotted against the days of intravenous feeding on a semilogarithmic graph.

J. O. passed a stool rich in minerals during the first day of intravenous feeding. Enemas were given on days 3 and 7. Two more stools were passed the last day of parenteral feeding, during which nausea and vomiting occurred. From these stools and the enemas on days 3 and 7, a total of 993 mg. of calcium, 706 mg. of phosphorus, 29 meq. of potassium and 1.7 gm. of nitrogen were recovered during seven days.

Bacteriologic Studies. The data obtained by culture of the stools and enemas are recorded in Table II. It will be seen that in five of

the eight cases studied (F. R., T. H., B. A., O. F., J. A.), there were increases of 3, 4, 75, 128 and 917-fold respectively, in the total number of viable aerobic bacteria recovered from the last as compared with the first enema. In one case (J. O.) there was no appreciable change in viable bacterial cells. In the other two cases (G. R. and H. I.) there was a decrease of 2 and 37-fold respectively in the second specimen.

The concentration of viable bacteria in the enema returns are shown on a semi-logarithmic scale in Chart I.

DISCUSSION

Chemical Data. The data yielded by these analyses of colonic washings are not clearly indicative of the status of intestinal tract physiology during total intravenous feeding. However, certain features seem worthy of comment.

One feels uncertain of the completeness of collection of colonic contents, and only gross differences can be fairly interpreted. In some instances materials may lie dormant in the gut for many days. Albright has stressed the so-called "lag period" in fecal excretion of calcium and phosphorus during metabolism experiments in which gross dietary alteration of these elements are made (8). Further evidence of this lag was our recovery of more than 3 gm. of calcium and 2 gm. of phosphorus in three stools passed postoperatively by a patient who had been on total intravenous feeding for eight days (9). Barium, too, may lie for long periods in an inactive colon. A patient with ileostomy for ulcerative colitis was given total intravenous feeding prior to reanastomosis. For many months nothing had been passed per rectum, but one month before operation a barium enema had been given to discern the colonic status. This was followed by several cleansing enemas. The first stool passed after reanastomosis contained considerable quantities of barium (9).

One feels uncertain also of the origin of colonic contents obtained early during the course of total intravenous feeding, due to the variable time of passage of food through the intestinal tract. These fecal contents probably are the result of food eaten prior to intravenous feeding. However, colonic contents obtained subsequently probably reflect material which has arrived by secretory activity during intrave-

nous feeding. Such material might result from upper intestinal tract juices³ which had not been reabsorbed, or secretions into the colon directly. The latter seems highly unlikely; previous studies with isolated pouches have shown little or no secretion by the colon of the elements under study here (7).

With these considerations in mind, one is struck by the small colonic contents of the patients in all three groups. Patients B. A. and H. I. in group I had no stools during the intravenous feeding periods of eight and seven days, respectively; and their colonic contents were negligible at the end of the periods. F. R., also in group I, had two spontaneous stools. That of day 2 may be discounted for reasons described above. That of day 8 may represent material secreted during the eight days of intravenous feeding.

Recovery of calcium, phosphorus, nitrogen and potassium in the washings of group II, who were given daily saline enemas, was slightly higher than in group I. The yield at the end of day 1 was not invariably greater than the average of subsequent days, though usually it was so.

In group III, who experienced some nausea and intestinal unrest at some time during their intravenous feeding, again with elimination of the fecal excreta of day I, one finds somewhat greater colonic contents than in group I.

The conclusion may be reached that, during total intravenous alimentation, in the absence of nausea or vomiting, and when enemata are not regularly administered, there are small quantities of calcium, phosphorus, nitrogen and potassium in the colon. Upper tract secretory activity is either greatly reduced, or the normal secretions are reabsorbed prior to reaching the colon or very promptly after reaching the colon. That there is intimate functional reflection on the small intestine by abnormalities in the colon and vice versa is well known. A patient (previously mentioned above) had an ileostomy; the colon healed and after a year reanastomosis was carried out. For three days

³ This includes gastric, intestinal and biliary fluids and saliva. We were unable to estimate the amount of saliva swallowed; the patients felt it was minimal. However, in nauseated patients, salivation was increased and probably considerable material was swallowed.

prior to reanastomosis and for eight days afterward, this patient was fed wholly by vein on a constant diet. The fluid contents emerging from the ileostomy contained slightly more than 200 mg. of phosphorus and from 100 to 150 mg. calcium per day. Subsequent to reanastomosis the patient began to have stools per rectum and the content of these stools fell steadily from 200 mg. phosphorus and 130 mg. calcium to 45 mg. phosphorus and 50 mg. calcium. During this same postoperative period, urinary calcium and phosphorus rose. Because of the altered physiology of the gut induced by the reanastomosis, there was less calcium and phosphorus excreted by the gut and more excreted via the urine. Whether the changed status resulted from the slower motility through the small intestine (and, hence, greater resorption), resorption of some calcium and phosphorus by the colon itself, or lessened secretion of these elements into the upper tract, we do not know. Nausea, or repeated enemata, seemed to act as stimuli to upper tract secretions, with the appearance of small amounts of calcium, phosphorus, nitrogen and potassium in the colon.⁴

Bacteriologic Data. Studies have been carried out by many earlier workers concerning the bacterial content of human feces. MacNeal, Latzer and Kerr (10), in studies on 14 subjects for several weeks, found considerable variation but estimated by direct counts and by centrifugation methods that an average of 33×10^{12} bacteria were excreted daily, which weighed dry 5.34 gm. and contained 0.585 gm. of nitrogen or 46% of the total fecal nitrogen. Matill and Hawk (11), in similar studies on two subjects, reported comparable results with an average daily excretion of 8.27 gm. of dried bacteria accounting for 53.9% of the fecal nitrogen.

No reports have been found of the total numbers of viable bacteria encountered. It is generally agreed, however, that viable bacteria account for only a small portion of the total bacterial mass. Moreover, it has been pointed out by Eggerth (12) and by Weiss and Rettger

⁴Relative quantities of these elements in the colonic contents followed a very rough pattern. The content of potassium rose somewhat more than the other elements in the patients who received daily enemas and those who were nauseated. In unpublished data from this laboratory, gastric juice has been found to contain considerable potassium (15 to 20 meq. per litre).

(13) that the majority of the viable bacterial cells in feces represent strictly anaerobic species; these would not have been detected by the methods used in the present study.

The pour plate method for bacterial counting used in this study is subject to considerable error. According to the criteria of Jennison and Wadsworth (14), however, this error was probably not greater than 20%.

The results of the bacterial counts presented above bring out several points of interest. It was surprising to find that the total number of viable aerobic bacteria in the first enema of patients T. H., J. O., F. R. and O. F. was about the same as the number in the whole stool which immediately preceded these enemas. In two of the patients, J. O. and F. R., the enema count was actually higher than the stool count, even though there were only traces of formed fecal material in the enema returns. The counts suggest either that the enema return contained unformed fecal material from high in the bowel, with more viable bacteria than the lower gut, or that viable bacteria occur in greater concentration on the mucosal surface of the bowel than in the mass of the stool itself.

The rise in the total number of viable aerobic bacteria recovered in the enema returns from five of the eight cases studied during the course of total intravenous alimentation is of considerable interest and implies that such bacterial species can thrive in the gut even in the absence of ingested food. The progressive bacterial increase in one patient (J. A.) who received daily enemas, further suggests that either increased secretion of the gastrointestinal tract as a result of these enemas, or the daily removal of bacterial metabolic products, promoted bacterial reproduction or survival.

The viable bacteria were in no case sufficiently abundant to account for any appreciable amount of the nitrogen observed. Since non-viable and anaerobic cells were not determined, however, it is possible that a considerable amount of nitrogen as well as the other elements measured might be of bacterial origin.

SUMMARY

The calcium, phosphorus, potassium and nitrogen and the bacterial content of spontaneous stools and enema washings have been studied

during periods of total intravenous feeding, and the following observations made:

1. During total intravenous alimentation, in the absence of nausea or vomiting, and when enemata are not regularly administered, there are small quantities of calcium, phosphorus, nitrogen and potassium in the colon. Upper tract secretory activity is either greatly reduced, or the normal secretions are reabsorbed prior to reaching the colon or very promptly after reaching the colon.
2. Frequent enemas or nausea and vomiting increased somewhat the amount of material reaching the colon during intravenous feeding.
3. In five of the eight cases studied, there was a rise in the total number of viable aerobic bacteria recovered in the enema returns, indicating that such bacterial species can thrive in the gut even in the absence of ingested food. In the single patient receiving daily enemas, there was a progressive rise in the aerobic bacterial count.

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THE BRITISH NATIONAL HEALTH SERVICE¹

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The National Health Service Act is one of three important measures for social security planned to come into operation in July, 1948. One of the other measures, which has still to be debated in Parliament, will sweep away the remnants of the Poor Law; will transfer the granting of financial aid from local to central government to be administered by a National Assistance Board; and will require the major local authorities to provide residential accommodation for the aged, infirm and others, together with special welfare services for handicapped persons, such as the blind. The third group of measures, already embodied in Acts of Parliament, deals with national insurance and certain welfare services which include family allowances, improved money benefits for sickness and unemployment, and more generous pensions in old age. A mid-day meal and milk are to be provided free for all children in state-aided schools, and milk and vitamin supplements, free or at greatly reduced prices, for pregnant and nursing women and for pre-school children.

The National Health Service is, in its main features, the logical outcome of a large number of public health measures that have been introduced during the past forty years. Indeed, the demand for such a service came in the first instance from the medical profession itself. The arrangements made during the recent world war for the co-ordination of hospitals in Britain showed the advantages to be gained from a well-planned hospital system, and the rising costs of medical care made evident the desirability of providing some form of national health service available to all the people. The decision to establish such a service was made by a Coalition Government and the outline of the proposed scheme was embodied in a White Paper in 1944. The Na-

¹A DeLamar Lecture delivered at the Johns Hopkins University, School of Hygiene and Public Health, October 3, 1947.

tional Health Service Act,² though passed by the present Government, differs in only a few—though important—respects from the White Paper scheme.

OUTLINE OF THE SERVICE

The Act places upon the Minister of Health a general duty "to promote a comprehensive health service for the improvement of the physical and mental health of the people of England and Wales, and for the prevention, diagnosis and treatment of illness." The Minister is, of course, assisted by a large body of technical and administrative civil servants and advisers and is responsible to, and liable to be called to account by, Parliament for all his actions or shortcomings. He will have a special advisory body known as the Central Health Services Council, consisting of a majority of professional persons together with others who have experience of hospital management, local government and mental health services. The presidents or chairmen of six of the principal medical organisations will be *ex officio* members. Standing committees of the Council will be appointed to advise on particular problems. In these ways practising members of the profession will be brought into direct contact with the regular work of the Ministry.

The Service will be available to every man, woman and child in the country. With some small exceptions there will be no fees or charges to the patient but it will be open to people, if they wish, to pay for additional amenities in certain cases—for instance, the extra cost of more expensive articles or appliances, or for additional privacy in hospitals (which in any event they will be able to obtain free if it is medically necessary).

HOSPITAL AND SPECIALIST SERVICES

The Act transfers to the Government the existing property, premises and equipment of all voluntary and public hospitals. Though the Minister of Health has a general duty to provide hospital and specialist services of all kinds, organised on a national scale, the actual administration of the services is delegated to regional hospital boards and to local hospital management committees.

² There are separate National Health Service Acts (1) for England and Wales and (2) for Scotland, but the differences are immaterial.

For this purpose, England and Wales are divided into fourteen regions, each based on a university medical school whose influence will, it is hoped, do much to promote higher standards of professional work throughout the regions. The hospital boards have now been appointed by the Minister after consultation with the universities, the medical profession, the local health authorities and the voluntary hospitals. Members with experience of the mental health services are included. These boards, as agents of the Minister and in collaboration with the teaching hospitals, will plan, and execute the plan for a co-ordinated hospital and specialist service for their regions. They are in process of appointing for each large hospital, or related group of hospitals, within their region, a local hospital management committee which will carry out the day-to-day management of the hospital under its control. In its task of appointing the members of these committees, the regional hospital board must consult with organisations representative of general medical practitioners, with the senior medical and dental staffs of the hospitals concerned, and with those local government bodies which have been given powers as local health authorities.

Hospitals or groups of hospitals that are designated as teaching hospitals are not under the control of the regional boards or local management committees. For each such hospital or group of hospitals a board of governors is being constituted which will include members nominated by the university, the regional board, and the senior staff of the hospital or hospitals concerned.

The endowments of the teaching hospitals will pass direct to the new boards of governors; the endowments of the other voluntary hospitals will be placed in a central Hospital Endowment Fund. After existing liabilities have been discharged, the capital value of the Fund will be apportioned among the regional boards and the management committees. The income of each portion may be used by these bodies as they think best and they may also draw on the capital for any approved purpose.

Specialists may give either whole or part time service and private practice will therefore still be open to them. Although they will be attached to the staff of particular hospitals they will receive their appointments from, and be the employees of, the regional hospital

boards or the board of governors in the case of teaching hospitals. The appointments will be made on the advice of an expert professional advisory committee and only after public advertisement of the vacancy in the manner customary for such positions in Great Britain.

Private accommodation will continue to be provided in hospitals for persons who desire to pay the whole cost of their treatment—subject to the over-riding right of other patients to be admitted to such accommodation if medical considerations urgently require it.

FAMILY DOCTOR SERVICE

To a large extent the family doctor service will be the logical development of the service created by the National Health Insurance Act of 1911. The health insurance scheme has been an important factor in securing a basic standard of medical service for the employed sections of the community and at the present time provides a service for nearly one half of the total population of the country. So successful has it been that as early as 1933 the British Medical Association in their "Proposals for a General Medical Service" recommended that health insurance should be extended to dependants of insured persons. Undoubtedly the experience of the past 36 years will greatly facilitate the administrative arrangements necessary for making the family doctor service available to all.

In the counties and in cities having the status of county boroughs, bodies known as local executive councils have been appointed to administer the general medical and dental services. Half of the members of these bodies are representative of the local doctors, dentists and pharmacists, and it is with these bodies and not with the Government or with local authorities that the family doctor will enter into contract. Any general practitioner will be entitled to take part in the Service in the area in which he is already practising—he may at the same time continue to treat privately for fees persons who are not on his public list. People will have free choice of doctor, subject to the doctor's own consent, and all the well established professional relationships between doctor and patient will be retained. It is hoped that the doctors will be real "family doctors", interested in the promotion of health and the prevention of ill-health just as in the treatment of the

sick. They will have all the available hospital, specialist and nursing help at their disposal.

A doctor will *not* be told where he must conduct his practice. Before he starts public practice in a fresh area he will have to obtain the consent of a central professional body known as the Medical Practices Committee, but consent can be withheld only on the ground that the area in question has already an ample supply of doctors while other areas are still under-doctored. A doctor aggrieved by any decision of the Committee may appeal to the Minister.

In future, practices that are wholly or partly within the National Health Service may not be bought or sold. However, as many doctors have invested considerable sums in the purchase of practices, the Government has decided to compensate them and a sum of £66 millions will be provided to meet the claims.

GROUP PRACTICE IN HEALTH CENTRES

There has been an increasing tendency in recent years for general practitioners to enter into group or partnership practices. Such practices differ from their American counterpart largely in that they are composed wholly of general practitioners, the individual members of the group professing some degree of specialisation within the competence of the family doctor. The Medical Planning Commission of the British Medical Association in its draft Interim Report gave support to this form of practice. While it is possible for such a group to practise without premises used in common, there is much to be said in favour of their sharing accommodation in what has come to be known as a health centre. In such a centre it is simple to provide more elaborate facilities for diagnosis and treatment, as well as secretarial and nursing assistance. In addition, some of the personal health services of the local health authority may usefully be based on such a centre and so make easier the participation in those services by the family doctor group. The advantage of having such a centre closely associated with a medical school and used for the teaching of medical students is so obvious as hardly to need mention. While doctors will continue to practise for some considerable time to come from their own professional premises, it is intended to experiment

largely in the provision of health centres. These centres will doubtless vary considerably in type, but if preventive and curative medicine are to blend in the new Service it is important that as many family doctors as possible should take part in the personal preventive health work for which the local health authorities are responsible.

DENTAL SERVICES

Here priority will be given to expectant mothers and young people. This will be done through the local health authorities' maternal and child welfare and school health services. For those who do not come within the priority classes a general dental service will be available, but such persons will not be able to obtain full dental care without waiting. Any dentist who wishes to take part in the public service will have the right to do so and, like the family doctor, will contract for service with the local executive council.

LOCAL HEALTH AUTHORITY SERVICES

The councils of counties and of those cities having the status of county boroughs will be responsible for the provision of health centres and for their maintenance and staffing—apart from the medical and dental staff who will be provided by the local executive councils. They will also have the duty—now only a power—of making adequate arrangements for maternal and child care (other than institutional), for domiciliary midwifery, for public health and home nursing, for ambulance services, and for vaccination against smallpox and immunisation against diphtheria. They are also empowered to make arrangements for the prevention of illness, the welfare and after care of persons suffering from illness or mental defectiveness, and for the provision of domestic help for households where such help is required on account of illness or other specified cause. County and county borough councils are responsible for education within their areas and, therefore, for the school health service.

The compulsory vaccination of infants is abolished. During recent years the compulsory element has become increasingly unpopular and much of the legislation of the Vaccination Acts is now unenforceable. It is expected that greater success will be achieved by methods similar to those which have already proved so successful in the case of

diphtheria immunisation, where a voluntary system has been established, based upon free facilities coupled with an intensive use of the methods of health education.

It will be seen that, though they will lose their hospitals, local health authorities will have their other functions considerably extended and will have many important duties to perform. Medical officers of health have been apt, during the past seventeen years, to spend too much time on problems of hospital planning and administration. They will in future be able to devote themselves to work that is more properly within their own sphere. In their epidemiological work they will be assisted by a nation-wide system of public health laboratories run by the Medical Research Council on behalf of the Ministry of Health. They will be the pivotal officers in relation to the various parts of the National Health Service. One of their main functions will be to secure proper co-ordination between the hospitals, the family doctors and the domiciliary services of the local health authorities.

MENTAL HEALTH SERVICE

This will be made an integral part of the National Health Service and it is expected that specialist staffs of all kinds will be shared by both mental and general hospitals. If progress in the prevention and treatment of mental illness is to be achieved, the sort of isolation from which mental hospitals have suffered in the past must disappear for good.

RESEARCH

The Minister is empowered to finance research into any matters relating to the causation, prevention, diagnosis or treatment of illness or mental defectiveness. No provision of this nature has existed heretofore and the new power should be very helpful. It is hoped to exercise it in association with the Medical Research Council.

THE COST OF THE NATIONAL HEALTH SERVICE

Initially the National Health Service is estimated to cost between £150 and £200 millions a year. To some extent this will be met by the weekly contributions levied under the National Insurance Scheme,

but as the funds available from this source will amount only to some £32 millions per year, the greater proportion of the cost will fall upon the Government, and will be met partly from central government funds and partly, though to a much smaller extent, by local taxes. It must be emphasised, however, that the Health Service is not an insurance scheme and every member of the public will have the benefit of the Service whatever may be his situation in relation to the National Insurance Scheme.

GENERAL

The Act provides the administrative structure of the Service; the working arrangements must be made by way of regulations laid before Parliament. The details of these regulations are being discussed at the present time with the various interested bodies. Considerable progress has been made with those parts of the Service that concern mainly hospitals and local health authorities. A good many matters are still outstanding as regards the medical profession. One important point is, of course, the remuneration of the family doctors. There is agreement on the range of such remuneration, as laid down in the report of the 'Spens Committee':

"In respect of a publicly organised health service, a scheme should be devised which will ensure that between 40 and 50 years of age approximately 50 per cent. of general practitioners receive net incomes of £1,300 and over, and which will also secure, so far as practicable, that between 40 and 50 years of age approximately three-quarters receive net incomes over £1,000, that approximately one-quarter receive net incomes over £1,600, that slightly less than 10 per cent. receive net incomes over £2,000 and that, in a small proportion of cases, it is possible to obtain net incomes of at least £2,500. By net income we mean gross income less such professional expenses as are allowed by the Inland Revenue for Income Tax purposes. Our recommendations are in terms of the 1939 value of money.

"Before 40 and after 50, practitioners should be remunerated at the rate applicable between 40 and 50 to the burden and responsibilities of practice which they are in fact carrying.

"On completion of resident hospital appointments a recently qualified practitioner should secure an initial net income of not less than £500 per annum, as an assistant to a doctor in general practice."

The Government's suggestion is that a small part of the remuneration should be by way of basic salary or guaranteed minimum and the

rest by way of capitation payment. Many doctors would prefer capitation payment only. The remuneration of specialists on hospital staffs has still to be negotiated, but it is anticipated that they will desire annual payment in respect of that portion of their time they give to hospital work. Dentists' remuneration, also, is at present under discussion.

It is impossible to say how many additional doctors will be required for the National Health Service. If group practice develops extensively and adequate secretarial and nursing help is provided for the group, it may be that the increase will be smaller than some people estimate. There will have to be a maximum number of persons on any doctor's list, though it will be difficult to keep the number within satisfactory limits in the early days.

Medical schools are training as many students as they are capable of taking, and the General Medical Council has issued new recommendations regarding the medical curriculum that should go some way towards fitting young graduates for the kind of family practice that it is hoped will be characteristic of the new service.

There is no doubt that, given goodwill on the part of the medical profession and an intelligent and flexible administration with adequate professional representation, the National Health Service can be made something really worth while no less for the doctors than for the people of Britain.

A FAMILIAL SPREAD OF VACCINIA WITH ONE DEATH

ISOLATION AND IDENTIFICATION OF THE VIRUS

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Generalized vaccinia in normal individuals and in eczematous children following vaccination is not rare, as evidenced by the numerous cases cited in the reports of Ellis in 1935 (1), Tedder in 1936 (2), McKhann and Ross in 1938 (3), and Jubb in 1943 (4). Less common, however, is the transmission of the infection to eczematous and normal individuals from other cases of generalized vaccinia, or from persons recently vaccinated (1, 2, 3, 4). In only a few of these cases has the etiological agent been isolated and identified (1, 2, 3, 5, 6). The present report concerns the spread of vaccinia from a recently vaccinated child to three other members of the family and the isolation and identification of the virus from two of these.

CASE HISTORIES

Case 1. A 3 year old negro male child (P. S.), who had a history of recurrent eczema since the age of 3 weeks, developed a skin eruption which the mother thought, at first, was an exacerbation of the eczema, even though he had been maintained on his restricted diet and had been free of lesions for approximately a year. In three days the character of the rash changed from a papular to a vesicular, weeping, generalized eruption accompanied by intense itching and slight fever. The child rapidly became extremely ill, and, realizing that this was different from his previous episodes of eczema, the mother brought him to the Harriet Lane Home of the Johns Hopkins Hospital three days after onset of his illness. Thirteen days before the onset of his skin lesions, a six months old sister, after routine vaccination on the upper arm, developed a "primary take" with vesiculation and umbilication of the lesion in seven days. This child shared a bed with her 41 year old mother and the patient.

On admission to the hospital on November 4, 1946, the patient was fairly well developed and nourished but severely dehydrated, semi-stuporous, although constantly scratching and rubbing his skin. Over the face, head, chest, and trunk there were round, slightly raised, discrete lesions 5 mm. in diameter. They were dry, umbilicated and encrusted for the most part, and a few were reddened and

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weeping although no pustules were observed. The skin over the arms and legs, particularly the elbows and knees, was hard, leathery and scaling. There was slight generalized lymphadenopathy. Examination of the lungs, heart, and abdomen revealed no abnormality. The temperature was 37°C. WBC 14,000 per cu. mm. Hgb. 9.5 gm. RBC 4.1 million per cu. mm. One blood culture yielded *Staph. aureus hemol.* while others were negative. In spite of penicillin and transfusions the temperature rose slightly to 37.8°C., the patient became progressively worse and died 36 hours after admission.

At autopsy (J. H. H. Path. No. 20254), the abnormal gross and microscopic findings were limited to the skin, spleen, and lymph nodes. The skin was covered by generalized discrete, scabbed papules, uniform in size, 5 to 7 mm. in diameter, with some tendency toward central umbilication. These were very numerous over the entire body and confluent over the chest and shoulders. There was complete sparing of the palmar and plantar surfaces as well as the axillary and inguinal folds. No lesions were found on the mucous membranes. The superficial lymph nodes of the neck and inguinal regions and the mesenteric nodes were enlarged and firm, but on section appeared normal. The axillary nodes, numbering about twelve on each side, were greatly enlarged, each group measuring about 8 by 5 cm. in diameter, ringed by a hyperemic zone. Smears of these areas showed no bacteria. The spleen weighed 50 gm. Its capsule was roughened by adhesions and the pulp firm, brick red in color, with several ill-defined areas of purplish mottling scattered irregularly throughout.

Microscopic sections of the skin lesions showed swelling and vacuolation of epithelial cells, with degeneration and disintegration of cells at the surface, and infiltration of mononuclear cells, neutrophilic and many eosinophilic polymorphonuclear cells. There was a similar infiltration in the dermis, particularly surrounding the blood vessels. "Ballooning" and hyperplasia of epithelial cells were also present in some of the sweat glands and hair follicles. Inclusion bodies of the Guarnieri type were seen in swollen epithelial cells in sections stained with Hematoxylin-Eosin and Giemsa (Fig. 1). The spleen on microscopic section showed acute splenic tumor and many eosinophils. Sections of the axillary lymph nodes showed marked lymphoid and macrophage hyperplasia, and infiltration with eosinophils. The anatomical diagnosis was as follows: Eczema vaccinatum. Hyperplasia of the superficial lymph nodes. Acute splenic tumor.

Case 2. Two days after the death of the child (P. S.), the 41 year old mother (G. S.) was admitted to the hospital with a history of "sores" of seven days duration. Sixteen days after the vaccination of her six months old daughter, the mother had noted a "sore lump" on her left temple and, following this, similar isolated lesions had appeared elsewhere. Chilly sensations had occurred the day after onset of these lesions, and since then she had felt "dragged-out." She had been in good health before the onset of this illness. Her past history was non-

contributory except for the fact that until puberty she had had episodes of eczema but since then had had no skin rashes. Three attempted vaccinations in the past resulted in "no takes." Family history revealed that her father had had eczema as a child and three of her 16 children had had infantile eczema.

On admission to the hospital, the temperature, pulse, and respirations were normal. She was well nourished and appeared in good health except for the presence of skin lesions. On the left temple there was a pustular, umbilicated and

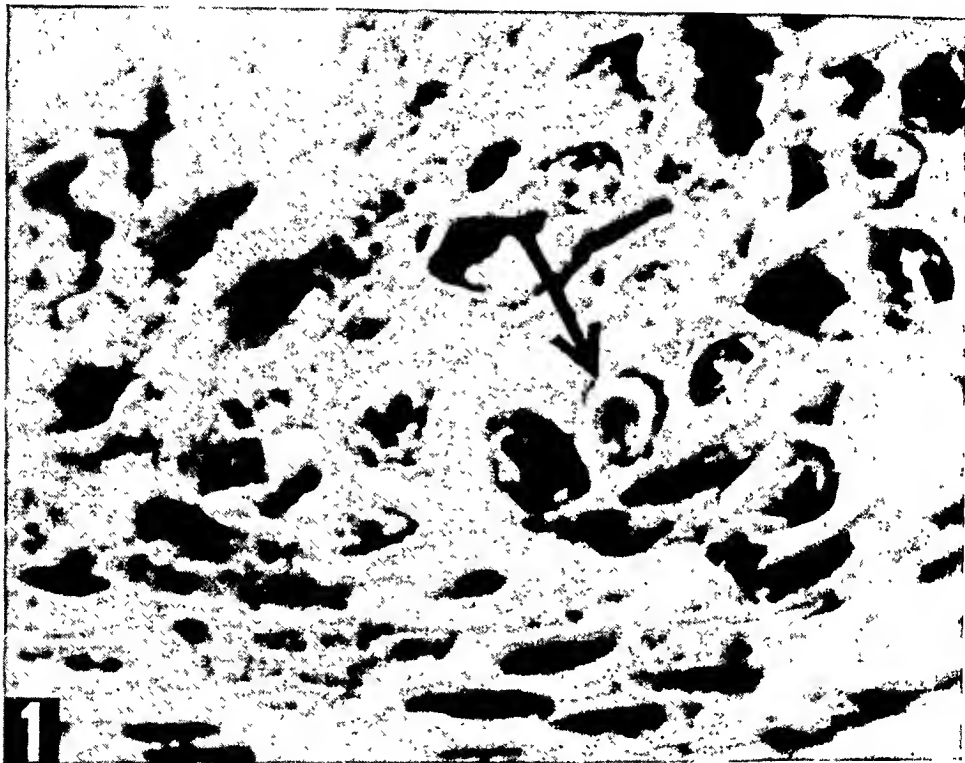


FIG. 1. SECTION OF SKIN TAKEN AT AUTOPSY FROM THE FATAL CASE OF VACCINIA SHOWING AN INCLUSION BODY IN AN EPITHELIAL CELL

ulcerated papule, 15 mm. in diameter, surrounded by induration. At the base of the left thumb there was a flatter, more umbilicated lesion, 5 mm. in diameter, resembling a vaccination "take" at about the 7th day. Similar isolated lesions were present on the right forearm and abdomen. Two papules, on the back and abdomen, previously treated with potassium permanganate, showed small clean central ulceration without evidence of secondary infection. Physical examination revealed no other abnormality.

Laboratory Data: Hgb. 12 gm. RBC 3 million per cu. mm. WBC 8000 per cu. mm., with 62% neutrophils, 31% lymphocytes, 5% monocytes, and 2%

eosinophils. The blood cultures were negative. Surface cultures of the temporal lesion revealed *Staph. aureus hemol.* and of the thumb lesion, *B. proteus* and *Staph. aureus hemol.*

The lesions cleared spontaneously without scarring in about one week and recovery was uneventful.

Case 3. At the time the mother was admitted to the hospital she was accompanied by a four year old son who had developed two lesions similar to hers at about the same time. (16 days after the vaccination of B. S.). These were described by the admitting physician as vesiculated, umbilicated, slightly pustular lesions, about 5 mm. in diameter. There were only two such lesions present, one on the abdomen and one in the right popliteal fossa. Unfortunately, this child was sent home at that time and remained unavailable for study.

EXPERIMENTAL METHODS AND RESULTS

At autopsy on P. S. a few of the fresher lesions on the thighs and chest were lightly scraped with a scalpel. The material thus obtained and small blocks of superficial skin removed from the chest wall were ground in a mortar with sand and diluted with physiological saline. After allowing the emulsion to settle to get rid of coarse particles, the cloudy supernatant was used as inoculum in an adult white male rabbit. Both corneas were lightly scratched and one drop of the emulsion put into the right eye, none in the left. The side of the same rabbit was shaved, lightly scratched, in a cross-hatched pattern, and the emulsion gently rubbed into the area. In three days there was an opacity of the right cornea with a small ulcer in its center. The left cornea was free of reaction and the original corneal scratch could not be seen. The skin, in the area of scarification, was reddened and indurated. Many vesicular papules were present. The vesicles then became umbilicated, confluent, and encrusted so that by the 6th day there was a fine crusting over almost the entire area of scarification.

Successive routine passages of the infectious agent thus isolated from P. S. were effected in rabbits every five to seven days by lightly scraping the encrusted area, collecting crust and lymph, diluting with saline, and using this as inoculum in the same manner as above. Each passage produced the reaction described above by the third or fourth day, the extent of vesiculation varying only slightly in various passages. Sixteen successive passages in rabbits were carried out in this manner. The crust and lymph harvested from certain passages were used in the

tests described below at the same time that routine transfer was done. The lymph and crust of numerous rabbit passages were stored in 80% glycerine in the icebox at 10°C. When last tested, after one month of storage, the potency had been maintained.

Sections of the lesions produced in rabbits by inoculation of scarified cornea, by rubbing emulsions into scarified skin, and by intracutaneous inoculation, were taken from numerous rabbit passages. These were fixed in Zenker's acetic acid solution and stained with Hematoxylin-Eosin and Giemsa. The lesion produced in the cornea was ulceration of the epithelium and hyperplasia of the marginal epithelium, with swelling and degeneration of these cells. Inclusion bodies were present in the marginal epithelial cells. There was thickening of the corneal lamellæ beneath the ulcer with large mononuclear, neutrophilic and eosinophilic polymorphonuclear cells. The lesions produced by intracutaneous inoculation of the rabbit skin were in every way comparable with those seen in the child's skin and described above. Those produced after scarification were similar, but showed more extensive superficial coagulative necrosis and less reaction in the dermis, although this was still appreciable. Inclusion bodies of the Guarnieri type were demonstrated in all the histological sections. Figure 2 shows these inclusions in a section of rabbit skin taken through a typical lesion 5 days after intracutaneous inoculation.

Filtration of emulsified crust through Seitz filters has been done at intervals, the bacterial free filtrate producing typical lesions by corneal and intracutaneous inoculation and by rubbing into the scarified skin of rabbits.

Titration of the virus have been done at intervals. The lymph and crust, obtained from routine passage rabbits, were weighed and, using 1 cc. of physiological saline per gram, diluted to 1:10. Serial dilutions were made from the supernatant of the 1:10 dilution after allowing it to stand for 15 minutes to prevent the carrying over of large particles of the emulsion to higher dilutions. Each dilution (0.1 cc.) was inoculated intracutaneously in the side and back of a rabbit. The end point of the titration was taken as the highest dilution producing erythema and edema with papule formation. The potency of the virus, by this method, has averaged from 1:4,000 to 1:10,000 and on one occasion was 1:100,000.

Bacterial cultures of routine transfer emulsion have at times revealed *Staph. aureus hemol.*, an unidentified gram positive bacillus, or non-hemolytic *Staph. aureus*. To test whether these organisms would produce the lesions generally obtained with the virus, each organism isolated from the emulsions was inoculated on the scarified skin of

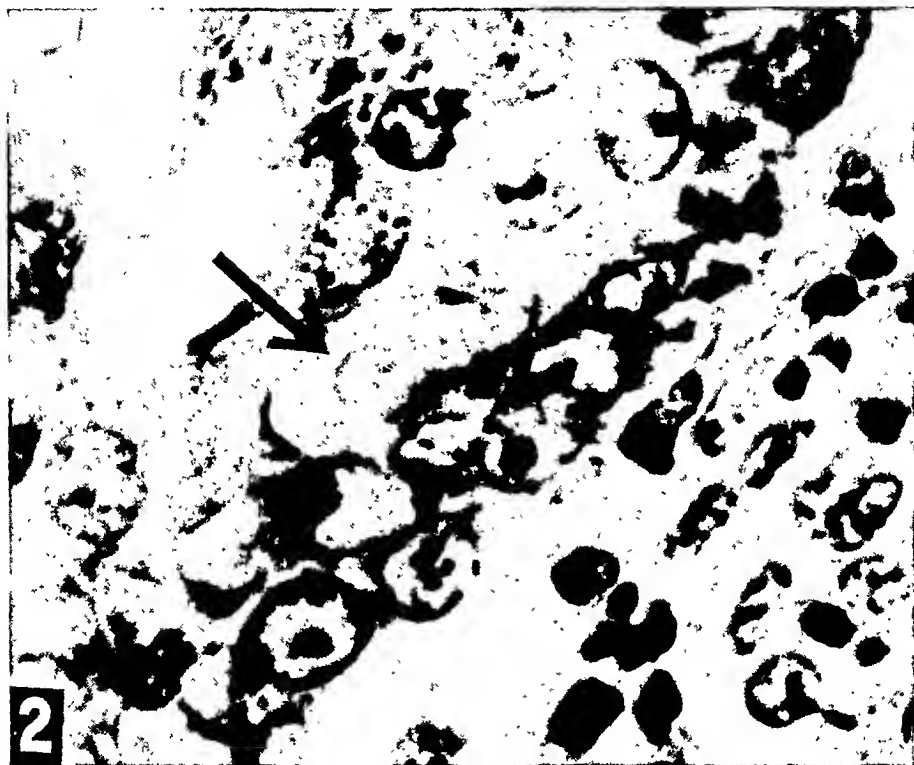


FIG. 2. SECTION OF RABBIT'S SKIN SHOWING AN INCLUSION BODY IN AN EPITHELIAL CELL

normal white rabbits of the same weight generally used. At no time were lesions produced by these organisms.

To further establish the identity of the virus isolated, a neutralization test was done in the following manner. The crust and lymph of the 13th rabbit passage were harvested, ground in a mortar and diluted 1:10 by weight with physiological saline. The emulsion was allowed to settle for 15 minutes and the supernatant serially diluted. One cc.

of each dilution was mixed with an equal amount of vaccinia immune human serum, and one cc. of the same dilutions of virus was mixed with an equal amount of normal human serum. The final dilutions of virus after the addition of the serum are given in Table I. The two sets of mixtures were placed in the incubator at 37°C. and shaken gently at 15 minute intervals. At the end of one hour at this temperature, 0.1 cc. of each dilution was inoculated intracutaneously into a rabbit shaved the previous day. All the immune serum-virus and normal serum-virus mixtures were inoculated into the same rabbit. The immune human serum used was obtained from an adult two months after an accelerated reaction from routine revaccination. This donor had never had smallpox or herpes. He had had measles and chicken pox as a child, but no other virus infections except common colds, and none of these in the past six months.

It was impossible to obtain normal (unvaccinated) adult serum, therefore the normal human serum used as control was a pooled serum secured from five young children who had never been vaccinated and who had not had smallpox.

Daily observations of the lesions produced by the intra-cutaneous inoculation of the serum-virus mixtures were recorded according to size of erythema, edema and papule formation and the presence of central vesiculation and umbilication (necrosis). For convenience in tabulating the results the reactions were later graded as follows:

- 0 No reaction.
- + Erythema and edema plus papule formation up to 5 mm. in diameter.
- ++ Erythema and edema plus papule formation over 5 mm. in diameter.
- +++ Erythema and edema plus papule formation over 5 mm. in diameter with central necrosis.

As can be seen from Table I, the titre of the virus (normal serum and virus) at this passage was at least 1:4,000. The immune serum completely neutralized the virus dilutions of 1:200 and higher, and partially neutralized the more concentrated dilutions of 1:20 and 1:100.

An attempt was made to isolate the virus from the lesions presented by the mother in the following manner. The superficial crust and lymph was removed from one of her lesions 13 days after its appear-

ance. This was emulsified and inoculated into the scarified cornea of a rabbit (Rabbit #1), resulting in a superficial ulceration of the cornea and conjunctival hyperemia in three days. Material from this lesion was transferred to the scarified cornea and skin of another rabbit (Rabbit #2). In three days a corneal ulcer and mild conjunctivitis were

TABLE I

Neutralization of child's rabbit passage virus by vaccinia immune human serum

DAYS		VIRUS TITRE						
		1:20	1:100	1:200	1:400	1:1,000	1:2,000	1:4,000
1	Vaccinia Immune Human Serum	+	+	0	0	0	0	0
	Normal Human Serum	++	+	+	+	0	0	0
2	Vaccinia Immune Human Serum	++	+	0	0	0	0	0
	Normal Human Serum	+++	+++	++	+	+	+	+
3	Vaccinia Immune Human Serum	++	+	0	0	0	0	0
	Normal Human Serum	+++	+++	++	++	+	+	+
4	Vaccinia Immune Human Serum	++	+	0	0	0	0	0
	Normal Human Serum	+++	+++	++	++	+	+	+

0, no reaction.

+, erythema and edema plus papule formation up to 5 mm. in diameter.

++, erythema and edema plus papule formation over 5 mm. in diameter without central necrosis.

+++, erythema and edema plus papule formation over 5 mm. in diameter with central necrosis.

present, but no skin lesions were produced. Further attempts to continue transfers of this strain in rabbits were unsuccessful.

Since corneal ulcers were produced in two of the rabbits, it was thought that the identity of the mother's lesions might be established by using the serum of these rabbits for neutralization tests against the virus isolated from her child, and by testing the immunity of these rabbits to direct inoculation of that same virus. Thus, approximately three months after Rabbits #1 and #2 were first inoculated, 50 cc.

of blood was removed from each. A neutralization test using each of these sera, and normal rabbit serum as control, was performed in the same manner as that described above for the test done with immune human serum. The virus used in this test was the 15th rabbit passage

TABLE II*

Neutralization of child's rabbit passage virus by serum of rabbits inoculated with mother's virus

DAYS	SERUM	VIRUS TITRE						
		1:20	1:100	1:200	1:400	1:1,000	1:2,000	1:4,000
1	Rabbit #1 (inoc. from Mother)	+	+	0	0	0	0	0
	Rabbit #2 (inoc. from Rabbit #1)	+	+	0	0	0	0	0
	Normal Rabbit	++	+	+	+	0	0	0
2	Rabbit #1	+	+	0	0	0	0	0
	Rabbit #2	+	+	0	0	0	0	0
	Normal Rabbit	++	++	+	+	+	+	0
3	Rabbit #1	+	+	0	0	0	0	0
	Rabbit #2	+	+	0	0	0	0	0
	Normal Rabbit	+++	+++	+++	++	++	++	+
4	Rabbit #1	+	+	0	0	0	0	0
	Rabbit #2	+	+	0	0	0	0	0
	Normal Rabbit	+++	+++	+++	++	++	++	++
5	Rabbit #1	+	+	0	0	0	0	0
	Rabbit #2	+	+	0	0	0	0	0
	Normal Rabbit	+++	+++	+++	++	++	++	++
7	Rabbit #1	+	0	0	0	0	0	0
	Rabbit #2	+	0	0	0	0	0	0
	Normal Rabbit	+++	+++	+++	++	++	++	+

* Grading as in Table I.

of the virus isolated from the child. The results (Table II) show that the titre of the virus was at least 1:4,000 and that both Rabbit #1 and Rabbit #2 sera completely neutralized the virus in dilutions of 1:200 and higher and partially neutralized the more concentrated virus.

Three months after they were originally inoculated, the two rabbits (Rabbits #1 and #2 above), in whom corneal ulcers were produced by the mother's virus, were tested for immunity by the intracutaneous injection of 0.1 cc. of each of the following dilutions of the child's rabbit passage virus: 1:10, 1:50, 1:100, 1:200, 1:500, 1:1,000 and 1:2,000. At the same time, the 3rd and 16th passage rabbits of the child's virus, 3 months and 3 weeks respectively, after original inoculation, were tested for immunity in the same manner. Normal rabbits, used as controls, were inoculated with the same dilutions of virus.

On the 1st and 2nd days after inoculation no difference between the supposedly immune and normal rabbits was seen. Positive reactions of similar intensities were obtained with each dilution up to 1:2,000. On the third day, however, all the reactions in the rabbits previously inoculated with the mother's or the child's virus showed less erythema and induration than the corresponding dilution in the normal rabbits. Each day thereafter, until the 6th, the lesions of the normal rabbits progressed while those of the other rabbits showed rapid healing. In addition, the normal rabbits' lesions produced by the 1:10, 1:50 and 1:100 dilutions showed central necrosis by the 4th day, while those of the other rabbits never showed necrosis, even in the lesion produced by the most concentrated inoculum. Therefore, it can be stated that the rabbits previously inoculated with the child's or the mother's virus showed partial immunity to the child's virus.

CONCLUSIONS

The three cases of generalized vaccinia presented in this report were probably contracted from the 6 months old vaccinated infant by direct contact. The incubation period in each instance, estimated from the date of vaccination of the 6 months old infant, who did not develop generalized vaccinia, to the time of appearance of lesions in the patients presented, was 13 days in the case of the 3 year old child who died, and 16 days in both the 41 year old mother and 4 year old brother. While it is possible that the mother's lesions and those of the 4 year old boy were contracted from the eczematous fatal case, it is unlikely, since all the cases developed within three days of each other. Mc-Khann and Ross (3) estimated the incubation period (from the time of inoculation of the source case to the time of appearance of lesions

in the contacts) in 26 cases of similar nature to be 9 to 23 days. Thus, if the three year old child who died was the source of the mother's lesions, the incubation period in her case would have been only 3 to 4 days and would be the shortest incubation period thus far recorded. It seems more reasonable to assume, therefore, that all the cases reported here had as their source the recently vaccinated child.

In all three cases the lesions were of similar nature, although of varied number and distribution, and all showed the typical umbilicated and encrusted lesions of vaccinia. Histological sections of the skin of the fatal case were similar to those described for vaccinia by Councilman and his coworkers (7) and showed many intracytoplasmic inclusion bodies. Eosinophils were abundant in the lesions of the case reported here and, although these were not described by Councilman, they have been noted by Howard and Perkins (8) and by Turnbull and McIntosh (9) in vaccinal lesions.

A filterable agent capable of producing vaccinia-like lesions in rabbits was isolated from the fatal case and carried through 16 successive rabbit passages. The role of bacteria in the production of the lesions in rabbits was excluded by filtration of the infectious material through Seitz filters, and by the failure to produce lesions in rabbits with bacteria isolated from contaminated virus emulsions.

Histological sections of skin lesions prepared from many rabbit passages were similar to those in the child, and similar intracytoplasmic inclusion bodies were demonstrated in the epithelial cells.

The virus isolated from the child was identified as vaccinia by neutralization tests employing known vaccinia immune serum. It is recognized, however, that this identification is incomplete, since no immune sera from other virus diseases were tested to see whether these would also neutralize the filterable infectious agent isolated.

Material obtained from the lesions of the mother produced corneal ulcers in rabbits, but continuous serial passage of this agent was unsuccessful. The sera from the rabbits showing the corneal lesions neutralized the virus isolated from the child, while normal rabbit serum did not. These rabbits also showed partial immunity to direct inoculation of the child's virus, responding in the same manner as routine child's virus passage rabbits did to reinoculation of the same virus. The neutralization of the child's virus by the sera of rabbits

inoculated with the mother's virus, and the partial immunity of these rabbits to direct inoculation of the child's virus, justify the conclusion that the same virus caused the infection in both and, from the evidence given above, that the virus is vaccinia.

SUMMARY

1. Three cases, one fatal, of generalized vaccinia occurring in the same family by contact with a recently vaccinated child are described.
2. The virus was isolated from two of these cases and identified as vaccinia by neutralization and immunity tests.
3. Sixteen successive rabbit passages were carried out with the virus isolated from the fatal case.
4. Intracytoplasmic inclusion bodies were demonstrated in the skin lesions of the fatal case and of inoculated rabbits.

The photomicrographs were made by Miss Marjorie Jackson.

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REDUCTION OF INTUSSUSCEPTION BY HYDROSTATIC PRESSURE

AN EXPERIMENTAL STUDY

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Despite widespread interest in the clinical aspects of intussusception, there have been few experimental studies of the problem. In 1893 Senn (1) found that manually produced intussusception in cats would reduce spontaneously if not fixed by sutures. He reduced experimental intussusception by insufflation of hydrogen gas and considered this an acceptable method of therapy in patients. D'Arcy Power (2) in a magnificently comprehensive Hunterian lecture (1897) described his own experimental work with guinea pigs, cats, and rabbits. He found difficulty in producing intussusception by means of cathartics and other pharmaceuticals. Intussusceptions which he produced manually were usually not dramatic in their results for his animals tolerated such intussusceptions fairly well. Nothnagel (3) (1904) found in cats and rabbits that steadily maintained faradic stimulation produced a tetanic contraction of bowel which occasionally caused the bowel to telescope into the segment distal to it. He was chiefly interested, as was Senn, in the mechanism of producing intussusception. Both of them noted that the intussusception develops "at the expense of the sheath," the leading point not altering. D'Arcy Power's interests were more far-reaching and his observations more acute than those of Nothnagel or Senn.

A great mass of clinical experience has been acquired over the past seventy-five years in the treatment of intussusception both by operation and by reduction using hydrostatic pressure. The bulk of this material demonstrates adequately to us by pragmatic test the safety and superiority of primary reduction by hydrostatic pressure (4). Two of the gravest objections still raised to the method are that gangrenous

bowel may be reduced and that bowel may perforate under pressure during reduction.

The present work was undertaken in order to determine experimentally the likelihood of such mishaps and to study the pathological changes and the clinical course of intussusception in animals.

Attempts to cause intussusception were made by painting the ileum with barium chloride and then invaginating the bowel with rubber-shod clamps. In some animals prostigmine was then injected into the corresponding mesenteric artery. The prostigmine did not produce any obvious effect upon the intussusception. Intussusceptions produced in this manner were irreducible by enema seven to eight hours later. We attributed this to the instrumental trauma to the bowel with resultant adherence of the adjacent coats.

The final procedure was as follows: The animals used were mongrel dogs weighing six to eight kilograms. Under nembutal anesthesia and with sterile precautions, the abdomen was opened through a right rectus incision. At a point about 14 cms. above the juncture of ileum and colon the terminal ileum was stimulated (Fig. 1) by a faradic current from an induction coil. While the bowel was still contracted strongly it was seized (Fig. 1) by rubber-shod forceps and inverted into the distal segment. If the entire intussusception was produced in this manner the serosal surfaces were sufficiently traumatized so that firm adhesions developed within twelve hours, making reduction impossible by hydrostatic pressure and difficult by direct manipulation. The intussusception, therefore, was initiated with the forceps, the forceps then replaced by a smooth glass rod (Fig. 2), and the intussusception completed with this instrument which was readily withdrawn without damage to the opposed serosal surfaces. Intussusceptions approximately 15 cm. in length were regularly produced. The level of the distal end of the intussusception was always marked by a silk suture in the serosa. The neck was usually at the ileocolic junction or just proximal to it.

Because the cecum of the dog is fixed to the posterior parietes there was usually not much progression of the intussusception. In two instances the intussusception became compound, developing a colocolic component which, by shortening the colon, permitted the intussuscepted ileum to prolapse through the anus (Fig. 3a & b). In two

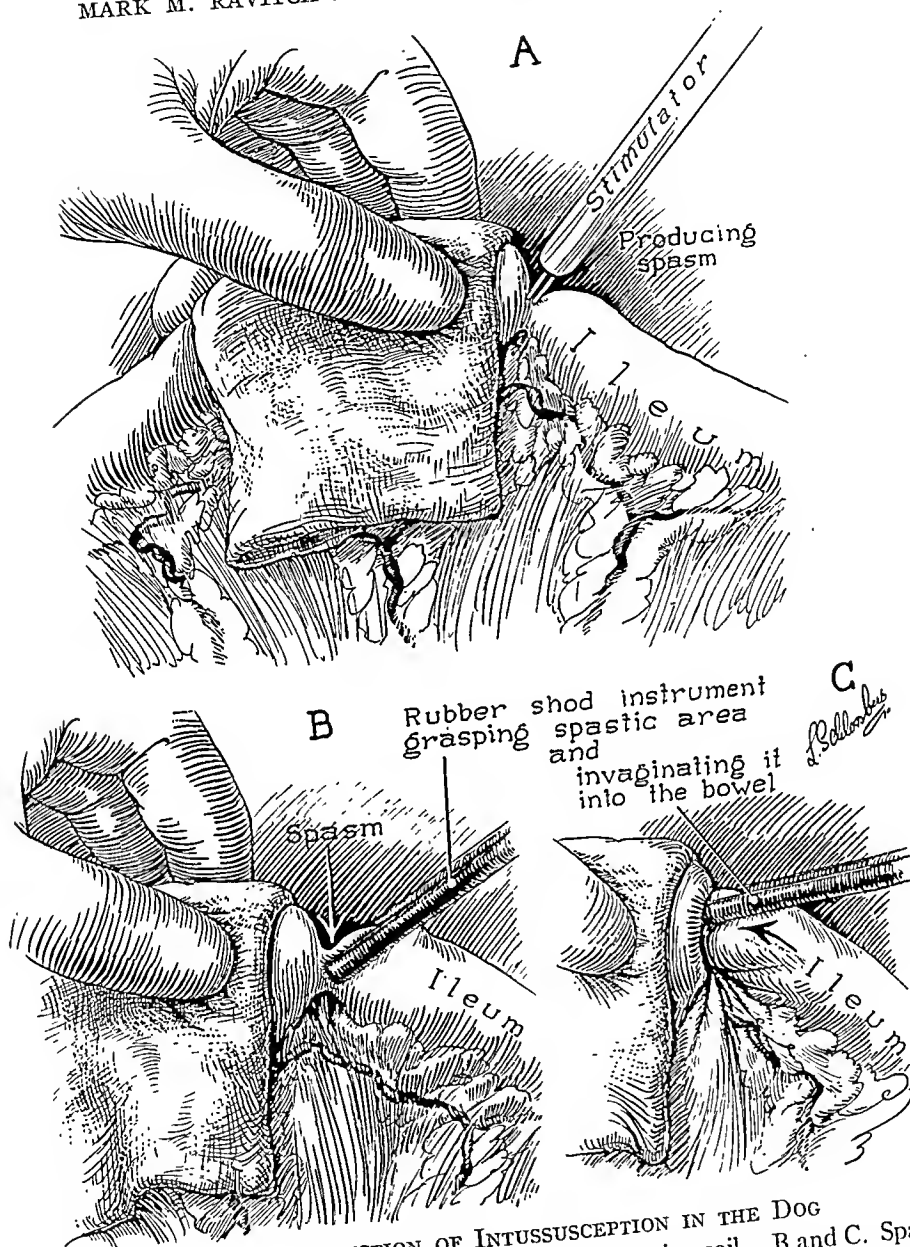


FIG. 1. PRODUCTION OF INTUSSUSCEPTION IN THE DOG
 A. Stimulation of ileum with current from an induction coil. B and C. Spastic segment is grasped with rubber-shod forceps and invaginated into distal bowel.

instances the intussusceptions reduced spontaneously. In two others the intussusceptions did not become gangrenous and the animals suffered from only partial intestinal obstruction. All of the other

animals gave clinical evidence of intussusception—anorexia, vomiting, passage of bloody mucous per rectum, and finally death unless the intussusception was reduced. In animals succumbing to intussusception the gross pathological picture was much like that in the human (Fig. 4). In a number of dogs six to eight hours after production of the intussusception 30 cc. of castor oil was given by stomach tube. Untreated animals usually survived over 48 hours. A total of fourteen

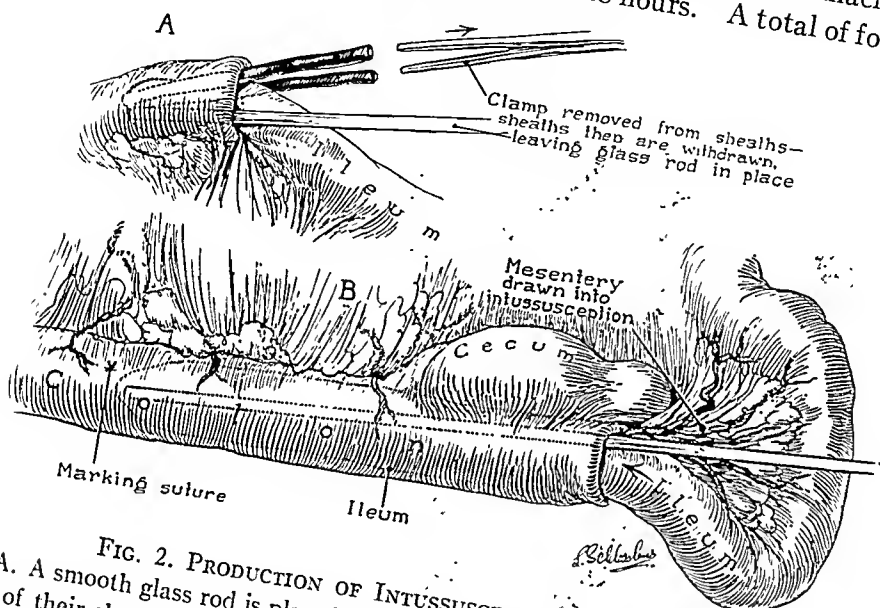


FIG. 2. PRODUCTION OF INTUSSUSCEPTION IN THE DOG

A. A smooth glass rod is placed alongside the forceps. The forceps are slipped out of their sheaths and the sheaths then removed. B. The intussusception is advanced 15 cms. at the expense of the recipient loop, the intussusciens. A silk suture marks the level of the intussusceptum.

dogs succumbed to unreduced intussusceptions. Six of these had intussusceptions produced by the earlier and more traumatic method with an average survival of 74 hours. Eight others had intussusception produced by the gentler method with the glass rod and survived an average of 88 hours.

After an interval of 18, 28, 38, or 48 hours each dog was anesthetized with ether and the abdomen opened. The serosal surface of the neck of the intussusception was cultured. A Foley bag catheter was then placed in the rectum, the balloon distended, and saline solution per-



FIG. 3. RM 39
A (left), intussusception gangrenous and prolapsed through anus at 47½ hours. B (right), compound intussusception. A colocolic intussusception was spontaneously produced.

mitted to enter from a height of three feet. The pressure was maintained for 5 minutes. If reduction was not achieved, the colon was allowed to evacuate, and after 5 minutes the process was repeated. If reduction was still not achieved a third attempt was made. If reduction was achieved, the serosal surface of the bowel was cultured again, this time at the level of the original apex of the intussusception. In



FIG. 4. RM 23. SPECIMEN FROM DOG WITH IRREDUCIBLE, GANGRENOUS INTUSSUSCEPTION AT 48 HOURS

either case, the abdomen was closed and the dog permitted to recover. The majority of the dogs whose intussusceptions were completely reduced had uneventful recoveries, the dogs in the 38 hour group experiencing the greatest difficulty. When the clinical signs of intussusception were present before reduction—anorexia, vomiting, listlessness,

DOG	DURA- TION OF INVAGI- NATION	CULTURE BEFORE REDUCTION	RESULT OF TREATMENT	CULTURE AFTER REDUCTION	FATE
RM 16	18	Sterile	Reduced 1 enema	Sterile	Anesthetic death
RM 17	18	Sterile	Reduced 1 enema	Gram + cocci	Survived
RM 18	18	Sterile	Reduced 1 enema	Sterile	Survived
RM 21	18	Sterile	Reduced 1 enema	Sterile	Survived
RM 19	28	Sterile	Reduced 1 enema	Sterile	Survived. Biop- sied at 9 days
RM 20	28	Gram + cocci	Reduced 2 enemas	Gram + cocci	Survived. Biop- sied at 7 days
RM 22	28	Sterile	Reduced 1 enema	Sterile	Survived. Biop- sied at 2 days
RM 29	38	E. coli	Reduced 3 enemas	E. coli, Strep. fecalis	Survived. Biop- sied at 10 days
RM 30	38	Sterile	Reduced 2 enemas	Strep. fecalis	Survived. Biop- sied at 8 days
RM 31	38	Sterile	Reduced 3 enemas	Sterile	Survived. Biop- sied at 6 days
RM 33	38	E. coli	Reduced 3 enemas	E. coli	Survived. Biop- sied at 4 days
RM 36	38	Streptococcus sali- varius	Reduced 3 enemas	Proteus vul- garis, Strep. sali- varius, Strep. equinus	Survived. Biop- sied at 2 days
RM 38	38	Sterile	Reduced 3 enemas	Gram—rods	Sacrificed immedi- ately after biopsy
RM 24	48	Gram + cocci	Irreducible	Sterile	Survived
RM 25	48			Sterile	Survived
RM 26	48				Chronic intussus- ception. Sur- vived. Biopsied at 7 days
RM 23	48	Gram + cocci	Irreducible		Anesthetic death
RM 27	48	Gram + cocci	Irreducible		Died at 96 hours
RM 28	48	Gram + cocci	Irreducible		Chronic intussus- ception. Sacri- ficed at 14 days
RM 32	48	Hemolytic Staph. albus	Irreducible		Anesthetic death
RM 34	48	Salmonella Para B (post-mortem)	No treatment		Died at 43 hours
RM 35	48	Strep. fecalis, E. coli	Irreducible		Died at 98 hours
RM 37	48	Cultures lost	Reduced 3 enemas		Survived
RM 39	48	Strep. fecalis, E. coli	No treatment		Died at 47½ hours
RM 40	48	Clostridium welchii	Irreducible		Died at 73 hours
RM 41	48	Strep. fecalis, E. coli	Irreducible		Died at 60 hours
RM 42	48	Streptococcus sali- varius	Irreducible		Died at 120 hours
RM 43	48	Strep. salivarius, E. coli	Irreducible		Died at 141 hours

FIG. 5. TABULATION OF EXPERIMENTAL RESULTS

passage of blood per rectum—they disappeared within 24 hours after reduction. Some dogs passed soft unformed stools for a few days. Otherwise, the dogs remained entirely normal to gross observation. Six of the ten dogs with irreducible intussusceptions at 48 hours showed a progression of their preoperative signs—vomiting, listlessness, tachycardia, passage of blood per rectum—and soon became unresponsive and moribund and died three or four days after production of the intussusception. Two of the 10 dogs with irreducible intussusceptions at 48 hours had chronic intussusception and showed little more than occasional vomiting and constipation, although one (RM 28) did show great weight loss by the end of two weeks. The remaining two of this group died from effects of anesthesia. The two dogs (RM 34 and RM 39) dying in under 48 hours had courses much the same as the other fatal cases described above. The two dogs showing spontaneous reductions at 48 hours (RM 24 and RM 25) and the dog successfully reduced at 48 hours (RM 37) recovered uneventfully.

It will be seen from the chart (Fig. 5) that whenever an intussusception could be reduced by hydrostatic pressure, the animals survived indefinitely (but for one which died under anesthesia). In no instance did any animal with a reduced intussusception have peritonitis, abscess, fistula, or any other complication to suggest that nonviable bowel might have been reduced. In no instance did bowel, reducible or irreducible, rupture. In the 18, 28, and 38 hour periods the intussusceptions were all reducible and all dogs survived. In the 48 hour group there were 13 dogs, excluding two whose intussusceptions had reduced spontaneously. Only one of these 13 intussusceptions was reducible by hydrostatic pressure. This dog survived. The other twelve were all irreducible by 3 feet of hydrostatic pressure and all 12 dogs died, except one in which the gangrenous intussusception was promptly resected for pathological study.

The culture reports are of special interest and are shown in Fig. 5. The following organisms were identified from these cultures, often several from one animal: *Escherichia coli*, *alpha Streptococcus fecalis*, *alpha Streptococcus salivarius*, *Clostridium welchii*, *Proteus vulgaris*, *alpha Streptococcus equinus*, hemolytic *Staphylococcus albus*, and *Salmonella para B*. As might be suspected, cultures were positive in two out of three dogs with intussusceptions 28 hours old or less, whereas

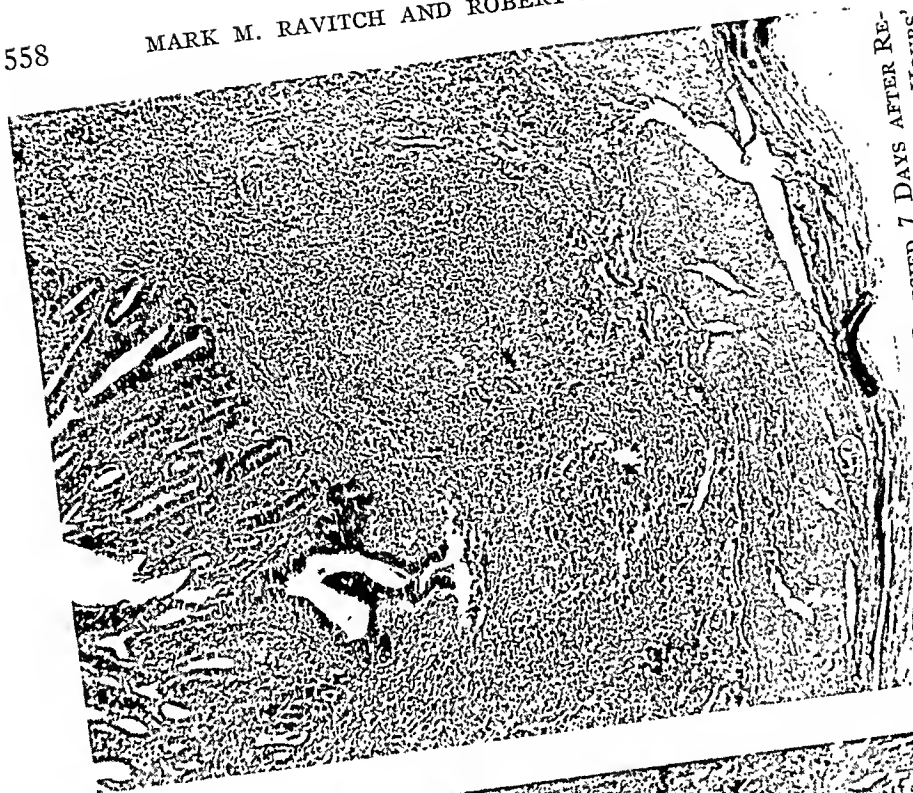


FIG. 7. RM 20 ($\times 60$). BIOPSED 7 DAYS AFTER RESECTION BY ENEMA OF AN INTUSSUSCEPTION OF 28 HOURS' DURATION. SEE TEXT

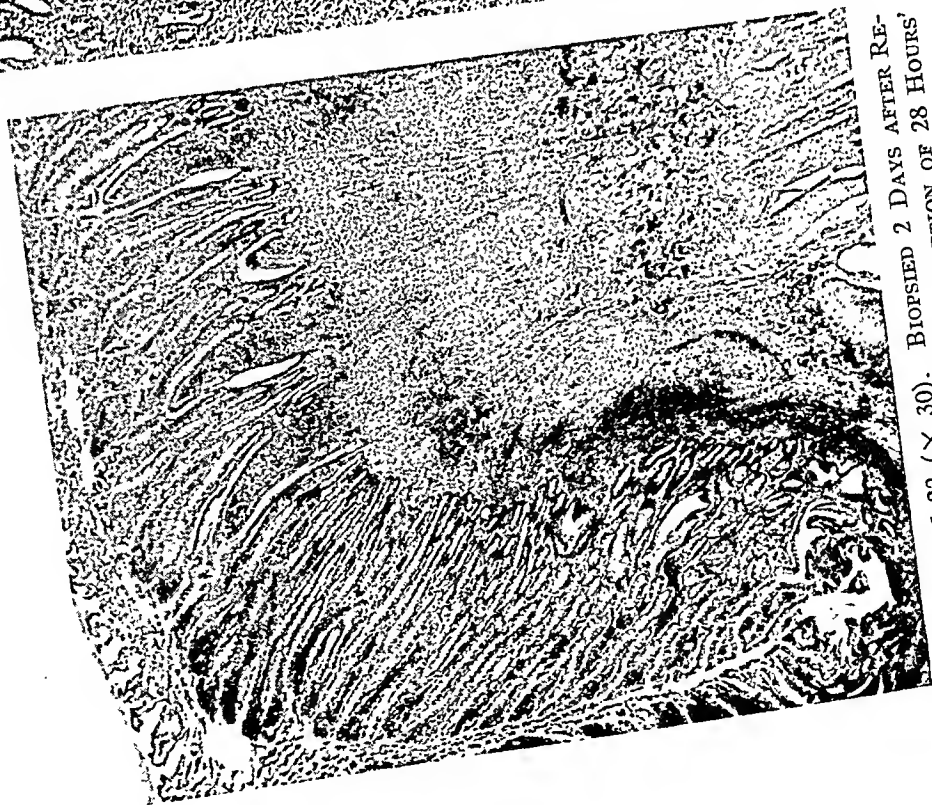


FIG. 6. RM 22 ($\times 30$). BIOPSED 2 DAYS AFTER RESECTION BY ENEMA OF AN INTUSSUSCEPTION OF 28 HOURS' DURATION. SEE TEXT



FIG. 8. RM 19 ($\times 60$). BIOPSIED 9 DAYS AFTER REDUCTION BY ENEMA
OF AN INTUSSUSCEPTION OF 28 HOURS' DURATION. SEE TEXT

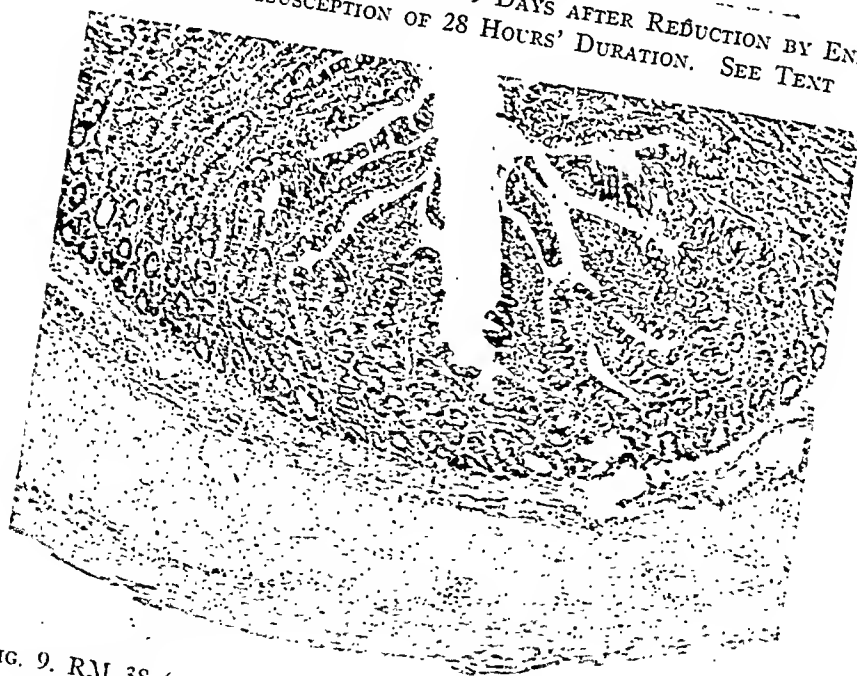


FIG. 9. RM 38 ($\times 60$). INTUSSUSCEPTION OF 38 HOURS' DURATION,
REDUCED BY ENEMA AND BIOPSIED AT ONCE. SEE TEXT

five out of six 38 hour old intussusceptions yielded positive cultures and twelve out of twelve 48 hour old intussusceptions yielded positive cultures. These results were anticipated by D'Arcy Power who suggested in 1897 (5) that "We do not yet know the exact course taken by the microorganisms as they pass through the wall of the bowel . . . but there seems to be no doubt that microorganisms begin to traverse intestinal wall when a loop of bowel has been constricted for a period of from 4 to 48 hours, and that the more completely the blood supply is arrested, the more rapidly they pass." It is striking that bowel as well preserved as that seen in Figs. 9, 10, and 11 permitted bacteria to pass through the wall. The clinical significance of these bacteriologic findings is apparent. They must account in part for the morbidity after operative reduction of intussusceptions—high fever (sometimes seen also after nonoperative reduction), abdominal abscesses, wound infections, postoperative adhesions with intestinal obstruction.

Pathological Features—I. The 28 hour group

RM 22. Intussusception of 28 hours' duration. Reduced. Cultures—all sterile before and after reduction. Biopsied 48 hours later (Fig. 6). The tips of the villi are sloughing and the villi are engorged with blood. The submucosa is tremendously edematous and hemorrhagic. The circular muscle is particularly edematous, fragmented, hemorrhagic, and infiltrated with round cells. The longitudinal muscle is relatively normal. The serosa is edematous and hemorrhagic and infiltrated with a few polymorphonuclear leucocytes among the round cells.

RM 20. Intussusception of 28 hours' duration. Reduced. Cultures—Gram positive cocci before and after reduction. Biopsied seven days later. In the gross the bowel was slightly thickened. Under the microscope (Fig. 7) the villi are found engorged with blood and round cells and a few polymorphonuclear leucocytes. The mucosa is flattened and the glands distorted. The thickening of the bowel is due more to the tremendous cellular infiltration of the submucosa than to edema. The inner circular muscle layer again appears glassy and infiltrated while the external longitudinal muscle layer is well preserved. No ulceration is seen but the villi seem thinned out. There is moderate serosal edema and round cell infiltration.

RM 19. Intussusception of 28 hours' duration. Reduced. Cultures—all cultures sterile. Biopsied nine days later. The bowel appeared normal in the gross. Under the microscope (Fig. 8) the epithelium is seen to have regenerated and appears normal but for increase in the number of goblet cells. The villi, although irregular, are tall. There is tremendous round cell infiltration of the

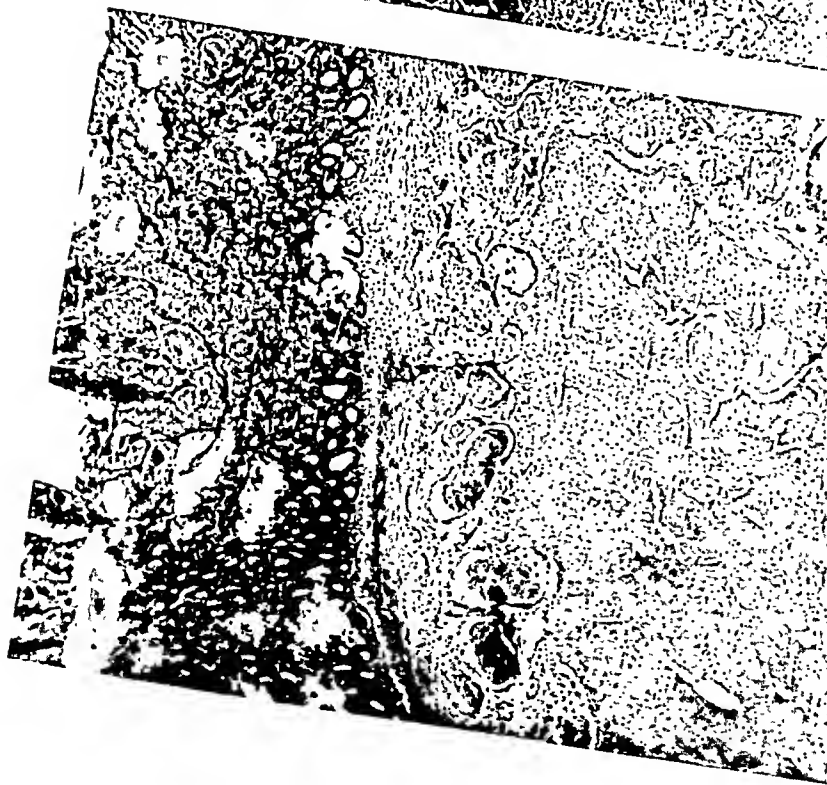


FIG. 10. RM 36 ($\times 60$). BIOPSED 2 DAYS AFTER REDUCTION BY ENEMA OF AN INTUSSUSCEPTION OF 38 HOURS' DURATION. SEE TEXT

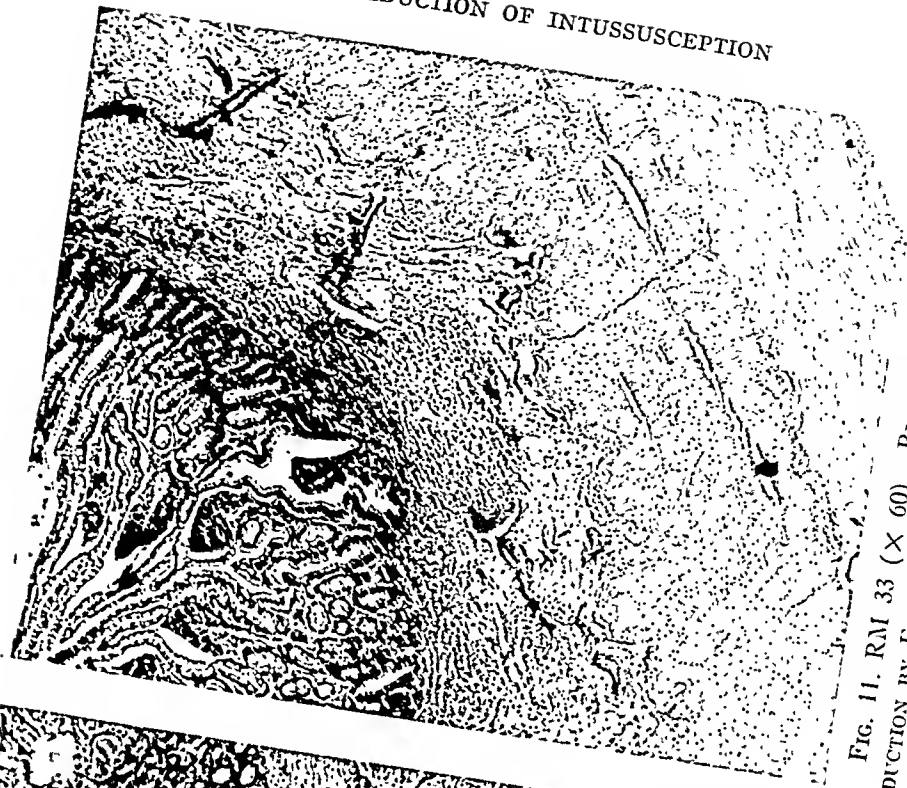


FIG. 11. RM 33 ($\times 60$). BIOPSED 4 DAYS AFTER REDUCTION BY ENEMA OF INTUSSUSCEPTION OF 38 HOURS' DURATION. SEE TEXT



FIG. 12. RM 31 ($\times 60$). BIOPSIED 6 DAYS AFTER REDUCTION BY ENEMA OF AN INTUSSUSCEPTION OF 38 HOURS' DURATION. SEE TEXT



FIG. 13. RM 30 ($\times 60$). BIOPSIED 8 DAYS AFTER REDUCTION BY ENEMA OF AN INTUSSUSCEPTION OF 38 HOURS' DURATION. SEE TEXT



FIG. 14. RM 29 ($\times 60$). BIOPSED 10 DAYS AFTER REDUCTION BY ENEMA OF AN INTUSSUSCEPTION OF 38 HOURS' DURATION. SEE TEXT

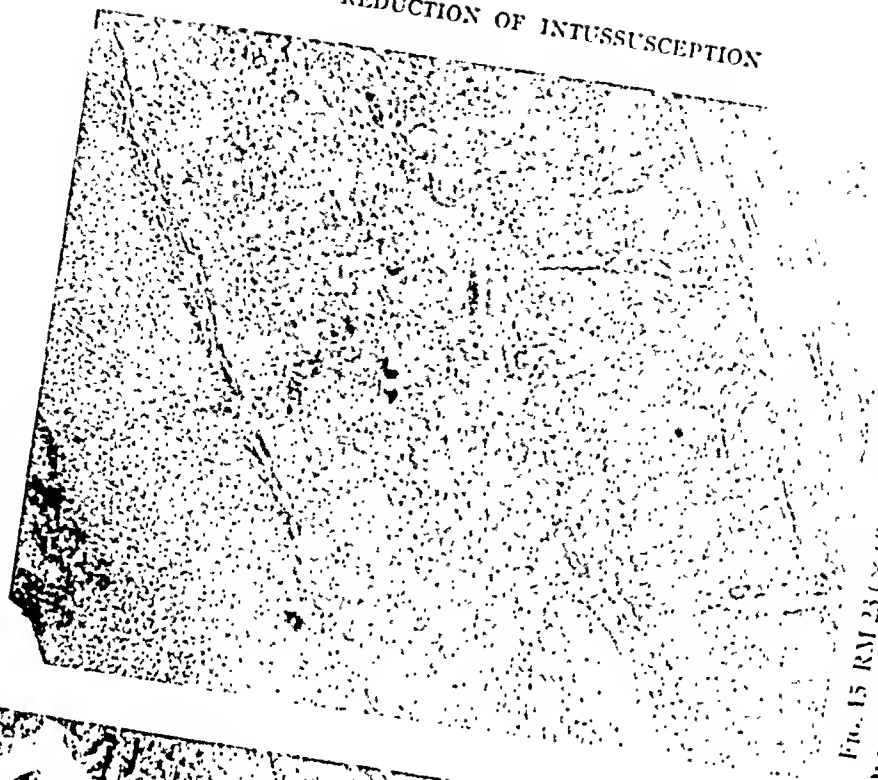


FIG. 15. RM 23 ($\times 60$). INTUSSUSCEPTION OF 48 HOURS' DURATION, REDUCED (SEE FIG. 4). SPECIMEN TAKEN AT ONSET. SEE TEXT

submucosa and numerous engorged vessels. There are a few polymorphonuclear leucocytes among the mononuclear cells. The submucosal lymphoid follicles are greatly enlarged and contain a few polymorphonuclear cells. The serosa is thickened and there are still a few round cells and plasma cells. The circular muscle layer is still abnormal, glassy, and infiltrated with many round cells.

Pathological Features—II. The 38 hour group

RM 38. Intussusception of 38 hours' duration. Reduced and the dog sacrificed at once. Cultures: before reduction—sterile; after reduction—gram negative rod-shaped bacilli. In the gross the serosa is edematous and beefy. The involved mucosa is darker than normal and sharply delineated. Under the microscope (Fig. 9) one sees cellular infiltration of the well-preserved mucosa. Goblet cells are numerous. Both muscle coats show severe changes, seen most plainly in the circular muscle coat, the viability of which is doubtful in some areas. There is fibrin on the serosa.

RM 36. Intussusception of 38 hours' duration. Reduced. Cultures: before reduction—alpha Streptococcus salivarius; after reduction—alpha Streptococcus salivarius, alpha Streptococcus equinus, Proteus vulgaris. Biopsied 48 hours later. The mucosa has partially sloughed and what remains is hemorrhagic (Fig. 10). There is tremendous engorgement of the vessels of all layers. The submucosa is greatly thickened. The internal circular muscle layer is infiltrated with round cells, greatly thickened, fragmented, and in sharp contrast to the slender, well-preserved outer longitudinal layer. The serosa is edematous and infiltrated.

RM 33. Intussusception of 38 hours' duration. Reduced. Cultures: before reduction—negative; after reduction—Escherichia coli. Biopsied after four days. The mucosa is injected and hemorrhagic but the villi are tall (Fig. 11). Goblet cells predominate. There is extensive submucosal infiltration and in this instance both inner and outer muscular coats show changes of moderate degree. Changes in this animal's intestine are not as marked as in many others.

RM 31. Intussusception of 38 hours' duration. Reduced. Cultures; negative. Biopsied six days later. The pathological changes (Fig. 12) are still striking; hemorrhage and engorgement have regressed but there is a heavy cellular infiltration most marked in the thickened submucosa and in the subserosa. Again goblet cells are prominent. The internal circular muscle layer stains poorly, is heavily infiltrated with round cells, and appears degenerated. The longitudinal muscle shows some cellular infiltration but the muscle fibers stand out sharply and the nuclei are well preserved.

RM 30. Intussusception of 38 hours' duration. Reduced. Culture: alpha Streptococcus fecalis. Biopsied eight days later (Fig. 13). The villi still show hemorrhage and an infiltration of mononuclear cells. The submucosa is thickened and heavily infiltrated with round cells. There is once more the usual difference between the muscle coats, the outer being well preserved, the inner not.

RM 29. Intussusception of 38 hours' duration. Reduced. Culture: *Escherichia coli*, alpha *Streptococcus fecalis*. Biopsied ten days later (Fig. 14). There is no more evidence of hemorrhage and engorgement. One still sees an abnormal number of goblet cells. There is some round cell infiltration of the submucosa. The inner circular muscle is almost hyaline. The outer coat shows some degenerative changes. The serosa is thickened and infiltrated with round cells and polymorphonuclear cells and shows beginning organization. Edema has entirely receded.

Pathological Features—III. Irreducible Intussusceptions

RM 23. Intussusception of 48 hours' duration. Irreducible. Animal sacrificed and specimen obtained. Culture: gram positive cocci. The bowel is almost entirely gangrenous (Fig. 15). The mucosa is unrecognizable. The submucosa is tremendously thickened. The inner circular muscle is completely disorganized while the outer longitudinal layer is startlingly preserved in contrast to the general destruction of the other layer. The serosa shows only edema. The vessels do not show clots or thrombi.

RM 5. Dog died at 56 hours of intussusception. Autopsied at once. The photomicrograph (Fig. 16) shows all three layers of this intussusception at the time of the animal's death. The changes are less extreme than in RM 23 (Fig. 17) but one can see clearly the mucosal hemorrhage and ulceration and the submucosal hemorrhage in the returning layer of the intussusceptum. These changes are much farther advanced and much more striking than they are in the inner or entering layer of the intussusception. The outermost layer, the intussusciptens, is seen to be relatively normal.

RM 23. Intussusception of 48 hours' duration. Irreducible. Animal sacrificed and specimen obtained. The photomicrograph (Fig. 17) shows a cross section of both layers of the intussusceptum (compare with Fig. 15). One sees clearly in the inner or entering loop the transformation of all the mucosal cells into mucous filled goblet cells, the source of the profuse mucoid discharge seen in patients with intussusception. The inner circular muscle coat is swollen to several times the thickness of the outer longitudinal muscle coat. Edema of the serosa is extreme. Passing now into the outer or returning layer, one finds it almost completely gangrenous. The muscular coats are almost completely destroyed. The mucosa is replaced by an amorphous mass of necrotic tissue and inflammatory cells and the submucosa is recognizable only because a few fibers of its connective tissue remain in the swollen necrotic layer.

Several features emerge from the pathological study of bowel recovering from intussusception. In these experiments the returning limb is regularly found severely damaged even to the point of gangrene at a time when the bowel in the entering limb is still viable. Under

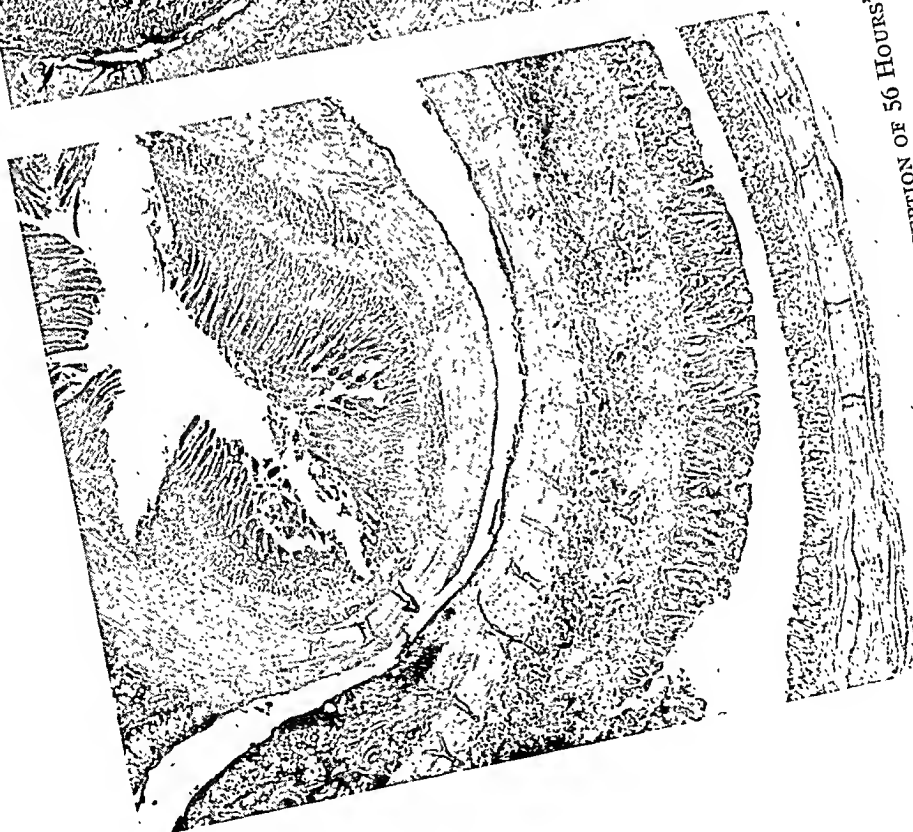


FIG. 16. RM 5 ($\times 12$). INTUSSUSCEPTION OF 56 HOURS'
DURATION
Dog died. Specimen taken at once. Cross section of
intussusceptum and intussusciptions. See text.

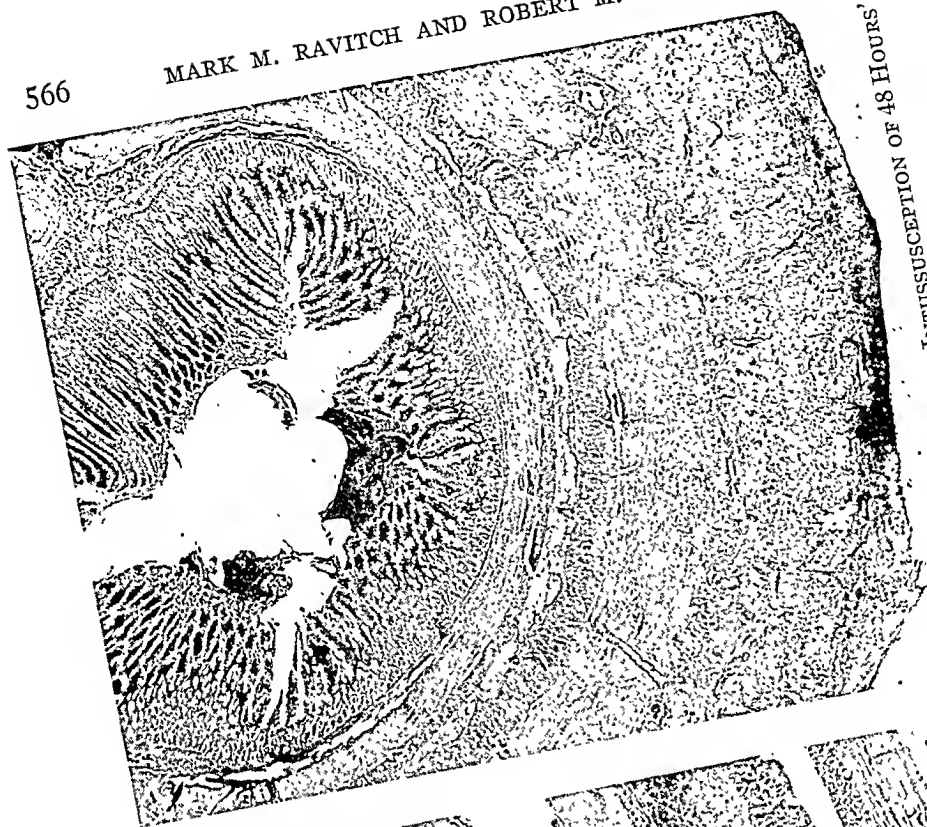


FIG. 17. RM 23 ($\times 12$). INTUSSUSCEPTION OF 48 HOURS'
DURATION
Specimen taken at once. Cross section of intussuscep-
tum. See text.

the conditions of this experiment and probably in intussusception beginning in the terminal ileum in patients, the returning limb is cut off from its circulation by the acute kinking of the bowel as it turns on itself at the apex of the intussusception. The pressure of edema should be the same on entering and returning layers, but the circulation of the returning layer is further interfered with by the U turns made at the apex of the intussusception and at the neck, effectively isolating this segment of bowel. Figs. 16 and 17 of dogs RM 5 and RM 23 illustrate this point. The changes caused by the condition are severe and still plain microscopically ten days after reduction (RM 29, Fig. 14). Hemorrhage and engorgement disappear first, then edema, and lastly cellular infiltration. The mucosa is frequently partially sloughed or eroded, but ulcers through to the submucosa were not seen. Despite this fact, pathogenic bacteria make their way through to the serosal surface even in bowel as well preserved as that of RM 20 (Fig. 7) in which the intussusception had been reduced after only 28 hours. This may be a partial explanation of the fever seen clinically after reduction and of the frequency of abscesses and infections after operative reductions. In none of the dogs in these experiments did any intra-abdominal or wound infections develop. There is a striking difference between the survival ability of inner circular and outer longitudinal muscular coats as graphically demonstrated in the photomicrograph of RM 23 (Fig. 15). The occurrence of large numbers of goblet cells in the intussuscepted bowel, remarked upon years ago by D'Arcy Power (5), would appear to explain the copious mucous seen in the currant jelly stools.

SUMMARY

1. A method is described for the experimental production of intussusception in the dog.
2. Intussusceptions so produced are usually fatal.
3. Intussusceptions of 18, 28, and 38 hours' duration in dogs can be reduced by hydrostatic pressure of three feet. Intussusceptions of 48 hours' duration are usually not reducible by hydrostatic pressure.
4. The pathologic features of intussusception are described.
5. The mechanism of the selective necrosis of the returning limb of the intussusceptum is discussed.

6. Cultures of the serosal surface of an intussusception containing viable bowel frequently showed pathogenic organisms, a possible explanation of the fever, distention, and adhesions seen in patients after reduction of an intussusception.

7. In no animal in any group was there rupture of the bowel.

8. In no animal was there reduction of an intussusception containing nonviable bowel.

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AUDIOMETRY WITH THE USE OF GALVANIC SKIN-RESISTANCE RESPONSE

A PRELIMINARY REPORT

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An objective diagnostic test of the threshold of auditory acuity has long been desired by the otologist and the audiologist. Although it has obvious uses in the entire range of clinical and research work relating to the hearing mechanism, such a test is particularly necessary to determine the hearing threshold of infants and young children and in making a differential diagnosis of a psychogenic overlay on organic hearing impairment and of malingering. With an instrument that records the activity of the sympathetic nervous system in response to acoustic stimuli, experiments at The Johns Hopkins School of Medicine have developed a technique of testing the hearing objectively. This instrument, developed under the direction of Dr. Curt P. Richter in the Psychobiologic Laboratory of the Phipps Psychiatric Clinic, gives a highly sensitive wave-record of galvanic skin-resistance response. The test procedure involves the stimulation of the subject by pure-tone signals from a standard audiometer, with the use of faradic shock, or other suitable stimuli, as a conditioning agent. The responses of the sympathetic nervous system to the test signals are amplified and charted with a recording milliammeter. From the recorded wave-response, a standard audiogram of pure-tone thresholds is plotted.

The test has proved useful for the entire range of the standard audiometer. Results obtained to date give evidence that the procedure is sound and can be standardized. Experimental work is going forward to refine the test procedure, to broaden the range of experimental subjects, and to adapt the test for speech audiometry. It is believed that in the field of physiologic research the technique of audiometry with the use of the galvanic skin-resistance response may be developed to the point where it will take the same part in the investigation of the human ear as the rôle played at present by the study of the cochlear potential in experimental animals.

PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD MEMORIAL HALL, FEBRUARY 9, 1948

Creatinuria in Man: The Role of Renal Tubule and Muscle Mass. DRs. KENNETH L. ZIERLER, JOHN W. MAGLADERY, BENJAMIN P. FOLK, JR., AND JOSEPH L. LILIENTHAL, JR. (Department of Medicine, Johns Hopkins Hospital).

It is convenient to consider creatinuria in terms of kidney function in which the load of creatine imposed upon the renal tubules exceeds the ability of the tubules to effect complete reabsorption of creatine. Creatinuria, then, may be due to increased tubular load (accelerated glomerular filtration rate or elevated plasma creatine concentration) or to reduced tubular reabsorption rate. Plasma creatine concentration, conceivably, may be elevated by administration of creatine, accelerated synthesis of creatine, extrusion of intracellular creatine, or inadequate disposition of creatine. Tubular reabsorption rate of creatine may be reduced as the result of several factors.

Personality Factors in Severe Poliomyelitis. DR. JACOB H. CONN. (Department of Psychiatry and Orthopedics, Johns Hopkins Hospital, and the Children's Hospital School (Respirator Unit), Baltimore, Maryland.)

The personalities and life situations of 15 patients with extensive, severe paralytic poliomyelitis in a Respirator Unit are described. There emerges a particular kind of person who behaves in a fixed, compulsive manner. This type of maladaptive, rigid personality development is to be differentiated from the "nervous tension" of average individuals under emotional stress, as well as from overt neurotic behavior. These patients appear to be happier, better adjusted, physically healthier and more active than the average person, but they lack spontaneous self-assertion; they have difficulty in criticizing others, and do not experience anger. They are overly conscientious, anxious to be accepted, appease others, and at the same time are striving to be independent.

Frustrations are met by becoming even more self-effacing, by adding to already heavy work loads, or by exercising excessively until there develops a pathological state of fatigue. The onset of the extensive paralysis generally occurs after months of strenuous effort during a period of relaxation and self-satisfaction, which follows the achievement of a greatly desired goal.

Use of Prefrontal Lobotomy for Intractable Pain. DR. FRANK J. OTENASEK. (Department of Neurosurgery, Johns Hopkins Hospital).

The author relates the result of prefrontal lobotomy in eleven patients who had intractable pain as a result of malignant tumors with or without metastases. Five cases are described in some detail. It is pointed out that the operation interrupts the pain pathways at a psychologic rather than a physiologic level. It takes away the affective components of a painful state by removing the patient's "reac-

tion to pain", while not objectively diminishing the actual perception of the painful sensation. All the subjects on whom the operation was performed had very brief life expectancies. When done in the painful states, such as tabetic crises or causalgia, in which life expectancy may be normal, the operation is likely to pave the way for an increase in knowledge of the functions of the frontal lobes.

Dr. Lawson Wilkins: I think it might be of some interest to recall that a few years ago Dr. Frank Ford and I described three children with exactly the same syndrome of inability to appreciate pain that you have found in these cases. At that time, we were not sure whether the lesion was lower down in the thalamus or was in the subcortical zone of the frontal lobes. Apparently complete inability to appreciate pain without any involvement of the sensory system may be caused by a congenital brain defect in children who are mentally normal and show no other neurological defect. One of our boys went around the streets with fractures in his feet from cobblestones having been dropped on them, but didn't bother to tell his mother. On one occasion he developed a ruptured appendix and peritoneal abscess which did not disturb him in the least.

Dr. Alfred Blalock: In connection with Dr. Otenasek's paper, there was one point which he undoubtedly did not have time to go into. He has talked to the surgical staff about the use of prefrontal lobotomy in patients who do not have pain, and I am sure he would be the first to say that the families of some of these people regard them as much greater liabilities postoperatively than they had regarded them before having this operative procedure. The only reason I mention this is that, although the use of this procedure may have perfectly definite indications, in some instances a patient may be a considerably greater liability than before. I wish Dr. Otenasek would comment upon this point.

Dr. Jacob H. Conn: I would like to add a word about the mechanism of pain. Dr. Freeman tells a very remarkable story of a young woman who had had a lobotomy. While lying in bed she heard the door rattle next door and knew there was an intruder there. The patient was aware that somebody was trying to break into the house, but she was not afraid because she couldn't anticipate what that person would do if he were successful in getting in. After lobotomy the ability to look ahead, to plan ahead, and to anticipate things is very defective. As Dr. Otenasek has very correctly pointed out, these patients feel the pain but cannot really anticipate further pain or dying.

Dr. Frank J. Otenasek: Dr. Blalock is entirely right in pointing out that lobotomy might make a patient a greater liability to his family and to the community than he was before. That is the reason, even in this beginning while we are feeling our way around, why we have operated on patients who have only a very short life expectancy and whose physical condition cannot be improved. From the beginning we have seemed to be justified in advocating the operation on patients with pain. In none of these cases was the family unhappy. The main contraindication was the patient's personality before operation. If the patient was psychotic, we felt it unwise to perform prefrontal lobotomy if there was any deterioration present. We did not operate in cases of overt homosexuality, etc.

Although the newspapers say it is possible to make a moral person out of an immoral one, under some circumstances that operation is much more likely to turn out poorly, and the patient becomes a real liability to the community. If we have to be fearful about releasing what few inhibitions a psychotic may have left, the operation is not done.

Changing Medical Care in our Changing National Life. DR. EDWARDS A. PARK.
(Department of Pathology, Johns Hopkins Hospital).

Dr. Parks' paper appeared in the Bulletin of the New York Academy of Medicine, December, 1947, and was reprinted in the Journal of Pediatrics, 31: 3, December, 1947.

Dr. Edwards A. Park: I speak on this subject with some embarrassment, together with some diffidence, because I am an amateur in this field. If what I have to say has any merit, it lies in the fact that, so far as I have been able, I have tried to think in a perfectly detached manner.

Dr. John T. King: Dr. Park claimed to speak as an amateur. I think he qualified practically as a professional. I shall speak as a real amateur in these matters.

As I understood him, Dr. Park pointed out in the early part of his talk that the medical profession has been busily engaged in protecting its vested interests. Toward the end of the talk he pointed out that certain plans for government intervention or insurance would greatly increase doctors' incomes. He assumes that doctors are ignorant of the prospect that certain proposed changes will increase their incomes. I doubt this, as I believe the doctors do understand this. There is an apparent contradiction in Dr. Park's remarks that the doctors are trying to protect their vested interests on the one hand, while rejecting a more remunerative plan on the other hand. Perhaps he can clarify this point.

Of course, we can sit here and discuss medical fees and prove very easily that certain indigent patients cannot afford diagnostic procedures and long hospital care at regular fees. But this is not the way the practice of medicine is carried on. In general, I would say that the average ethical doctor approaches these matters very much as this hospital does. If the patient can afford regular fees, he pays them. If he can pay part of the fees, he does so. If he is able to pay nothing the doctor will carry him. Now, when you undertake to subsidize doctors either by salaries, or by health insurance, or what not, you take away a real vested interest in which doctors have taken pride. That is to say, you pay the doctor for everything he does no matter how poorly the individual is being treated. That, it seems to me, would be a serious blow to the doctor's morale. You can pay a doctor a fee to go to an indigent patient, but you cannot make him give that patient good care. The type of care he gives will be determined more by his interest in the patient, by his pride in his profession, and by whatever satisfaction he may obtain in treating indigent patients. My own feeling is that revolutionary changes in the practice of medicine are not advisable. Possibly some branch of government should help our hospitals and medical schools over the crisis that exists now when it is so difficult to obtain private funds. I agree with the plan of

subsidized laboratories to carry on expensive tests which the patient and doctor cannot afford, such as have been established by our local Health Department. Moreover, some public source might very well furnish expensive material necessary for treatment. For example, collections are now being taken in Baltimore for the purchase of streptomycin. With all the billions of dollars for the care of the sick and indigent in this country, we do not yet find streptomycin available. We do not even have sufficient funds to enable the hospitals to pay for penicillin. Subsidies of this sort, it seems to me, are a direct concern of the government and a form of aid to which few could take exception.

Dr. Park: I didn't mean to make a contradiction. I, too, expressed surprise that the medical profession in general oppose health insurance as obstinately as they do—that is, compulsory health insurance—because it would be so much to the advantage of the average doctor if the impecunious patient could pay his bill. I think it is true that we doctors are ordinary human beings, and as such see things from our own interest. I think that if we are honest with ourselves, we all of us will admit that, like others in the struggle for existence, we are fundamentally selfish in our outlook. I think that the opposition to any change in medical care has its origin very largely in the fear that our economic interests will be jeopardized or our interests, in an economic sense, will be limited. I don't believe that the sliding fee-for-service is at all essential, so far as the preservation of the relationship between the doctor and the patient is concerned. Certainly that has not been the situation in Sweden where government medicine affects probably half of the population and at least half the physicians. Professor Lichtenstein was very careful to point out the fact that state medicine didn't interfere with the sanctity of the physician-patient relationship. The physician-patient relationship is determined by a spirit within and is not determined by economic considerations, in my opinion. I don't know that I can say any more than this, except that in regard to this last point I must take a view which is contrary to that expressed by Dr. King.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Atlas of Cardiovascular Diseases, Correlation of Clinical Electrocardiography and Cardiac Roentgenology with Clinical History and Autopsy Findings. By IRVING J. TREIGER. Illus. 180 pp. \$10.00. C. V. Mosby Company, St. Louis, Missouri, 1947.

This is a handsomely printed and illustrated atlas in which brief summaries of the clinical examination and course are combined with reproductions of the teleroentgenogram, electrocardiogram, and gross pathological specimen, with a summary of the autopsy findings. The atlas attempts to cover all types of heart disease, but there is considerable variation in the thoroughness with which various topics are discussed. For instance, the subject of patent ductus arteriosus is covered by one short theoretical paragraph without clinical descriptive material, and with one illustration of a heart at autopsy. Coarctation of the aorta is likewise briefly treated. Syphilitic aortic aneurysm is dealt with by two clinical records with accompanying x-ray and electrocardiographic findings of patients who did not come to autopsy; four illustrations of autopsy material on other cases are also given. The atlas would probably have definite teaching value for medical students, but the material is probably too limited in scope to contribute much valuable information to the well-trained clinician.

C.B.T.

Calcific Disease of the Aortic Valve. By HOWARD T. KARSNER AND SIMON KOLETSKY. Illus. 111 pp. \$5.00 J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

This book is the result of a detailed investigation of 200 cases of calcific disease of the aortic valve, which was carried out "in order to learn whether the lesion is sclerotic, degenerative and involutinal, or whether it is basically inflammatory". The evidence collected points strongly to the view that the lesion originates in inflammation and that the inflammation is usually, if not always, a manifestation of rheumatic fever. While a major portion of the book is devoted to the pathology of this condition, careful attention is given to predisposing factors, clinical features, and a historical review of the entire subject. The book is clearly written and presents a wide range of material from the literature. It is an authoritative account of calcific disease of the aortic valve which should be on the reference shelf of every internist concerned with cardiac disease.

C.B.T.

Congenital Malformations of the Heart. By HARRY J. LAMM. Pp. 612. \$10.00. Commonwealth Fund, New York, New York, 1945.

This book is a notable reference in the literature. It gives a new collection of cases and in the directions of interpretation for each literature. Dr. Thompson makes the accurate identification and a general analysis of the different cases for the vitamin. The careful description of these elements for learning is useful to compare the latest many years and an experience in the use of the subject to the studies and variety of carefully studied elements.

The book is divided into three parts. In the first, the author discusses the embryology and physiology of the postnatal ear, as well as the pathogenesis of clinical disorders. It is to these latter that Dr. Yarnik's contributions have particularly contributed. The second part is devoted to the clinical development occurring at some specific stage in the infant's life. Deafness is not there pictured as the least of infantile ear malfunctions, the only type rarely afflicted except as it may be indirectly influenced by the child's general health. The dynamic consequences of the malfunctions are referred to the ear's function in memory of the voice in least children as far as is indicated by the available data. Dr. Yarnik places great emphasis on the factor of heredity in the

In the past, analysis of congenital cardiac malformations has been primarily concerned with intra-ventricular differentiation of the myocardium, and the development of the coronary arteries. In contrast, the embryology of the pulmonary arteries has been poorly understood. The purpose of this paper is to review the embryology of the pulmonary arteries. The second portion of the paper is devoted to the description of the malformations which deprive the fetus of an adequate pulmonary circulation and to the management of these. Here are included tetralogy of Fallot, pulmonary stenosis or atresia, and the less common forms of aortic atresia and transposition of the great vessels. In part three are described malformations which are not incompatible with oxygen supply sufficient for growth, including patent ductus arteriosus, septal defects, and malrotation of the aorta. In part four the author offers her recommendations as to handling the therapy of these abnormalities, including the medical aspects of surgical treatment of congenital pulmonary stenosis.

The book is intended as a volume of reference. As written, each of the four sections is more or less complete in itself. While this has resulted in some unavoidable repetition and has increased the size of a large book, it has also increased the usefulness of the volume for its intended purpose. The various abnormalities are likewise described in colored diagrams which present both anatomical and physiological information regarding the abnormality concerned.

With the design of surgical procedures for the correction of congenital abnormalities of the heart or great vessels, or for the relief of important dynamic consequences of these lesions, some types have been removed from the category of irreversible cardiac disease. If stimulus were needed, these possibilities challenge the physician to the greatest accuracy of diagnosis of congenital cardiac defects. In achieving this he will lean heavily upon Dr. Taussig's recorded experience, by which knowledge of the field of congenital heart disease has been so usefully enriched.

E.C.A.

Diseases of the Nose, Throat, and Ear. 9th Edition. By WILLIAM L. BALLENGER, HOWARD C. BALLENGER, AND JOHN J. BALLENGER. Illus. 993 pp. \$12.50. Lea and Febiger, Philadelphia, Pennsylvania, 1947.

This is the ninth edition of a well known and excellently edited textbook on otorhinolaryngology. A chapter on headaches and a new section on plastic surgery of the nose have been added to the previous edition, and the texts of the other chapters have been thoroughly revised and brought up to date. In its present form, this book is undoubtedly one of the finest and most complete in the field of otolaryngology. The new chapters are quite comprehensive. The illustrations of the surgical procedure in rhinoplasty are among the best that have been produced in any textbook. The coverage of all the latest advances in therapy is comprehensive and detailed.

The bibliography in each chapter makes it an excellent reference book as well as a textbook. It is a pleasure to review a book with so many references on each subject. Unfortunately, bibliographies are all too frequently incomplete or even absent in the modern editions of textbooks on Ear, Nose and Throat. This book is an excellent standard work for those students specializing in the field of otorhinolaryngology.

J. E. B.

Nutrition in Health and Disease. 10th Edition. By LENNA F. COOPER, EDITH M. BARBER, AND HELEN S. MITCHELL. Illus. 729 pp. \$4.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

Nutrition in Health and Disease is written primarily as a textbook for student nurses, but it is also planned to serve as a reference book for the dietitian, graduate nurse, and homemaker.

Part I discusses the general principles of nutrition. New chapters on planning family dietaries to meet the recommended dietary allowances and on Nutrition for Adults—young and old—are valuable additions. The infant feeding schedule, however, seems unnecessarily restricted by present day standards.

Part II covers diet in disease. All therapeutic diets are discussed as modifications of the normal diet, as it is felt that a well balanced diet is the basic principle for all dietary prescriptions. However, many of the sample diets do not carry out this precept.

The third section is devoted to the basic foods and their preparation for the patient. The inclusion of recipes in a textbook of this type seems of questionable value when so few recipes in each category can be included.

The tables of food values have been completely revised and simplified in Part IV. A short method of calculating dietaries has been included. This will facilitate calculations when accuracy is not a primary factor.

In an attempt to cover all phases of the subject much material has been only mentioned, which limits the student's understanding and appreciation. As stated in the preface the text is primarily for the student nurse and it may well be used as a basic text but should be amply supplemented by other references.

B. C.

Preoperative and Postoperative Care. By WILLIAM J. TROTTER and FREDERICK B. WAGNER, JR. Illus. 305 pp. \$6.00. F. A. Davis Company, Philadelphia, Pennsylvania, 1947.

This book is essentially an intern's manual on pre-operative study and treatment and postoperative care. The largest portion is concerned with the general surgical patient. Essentially, it is a check list of pre-operative and, as such, should be helpful in assuring the completion of pertinent studies. There is very little statement of the rational basis for the various pre-operative. There is also a great deal of repetition. Standard post-operative procedures are appropriately detailed. The section on the passage of the Miller-Abbott tube is unusually helpful. There are special sections on the management of the common surgical topicals and the neurological patient. That on the unhealed patient is unusually good.

W. L. G.

Sexual Behavior in the Human Male. By ALFRED C. KINSEY, WARDEN H. POMEROY, AND C. E. MARTIN. 304 and XV pages. \$6.50. W. B. Saunders Company, New York, New York, 1948.

This study brings a monumental contribution to the knowledge of human sexual behavior. Nothing comparable has been achieved before. The sexual histories of 12,000 persons have been secured, 5,500 are reported etc., 10,000 is the goal. The investigation, under the leadership of Alfred C. Kinsey, Professor of Zoology, Indiana University, has been supported by the National Research Council's Committee for Research on Problems of Sex, by means of funds contributed by the Medical Division of The Rockefeller Foundation.

The authors present in this book, the first of a series, an account of the sexual experiences of 5,500 American men and boys, amounting, as the reviewer estimates it, to twenty million or more such events. This is a vastly larger sample of human sexual behavior than has ever been studied and reported before; the information has been obtained in a thorough fashion by the technique of individual confidential interview, and the data have been analyzed by appropriate statistical procedures, and with great practical good sense. The focus of attention is physiological, and the overall investigation, of which this is the first report, has been formulated as a "taxonomic" study of the sexual behavior of the human species in the American environment. The physiological orgasm is taken as the unit for counting. Emotional and moralistic issues have been subordinated to what is considered a "scientific," fact-finding survey, and this natural-history approach has doubtless been a great help in gathering and evaluating information. Observations on emotional reactions and attitudes, which would also have a scientific value and would be of particular interest to the psychiatrist, have received some attention, and the data obtained have provided illuminating side lights on such attitudes, particularly as influenced by one's social class.

With appropriate statistical corrections for age distribution, the American male has been calculated from these data to have approximately 3 sexual orgasms per week. This total outlet is variously distributed among masturbation, nocturnal emissions, heterosexual petting, heterosexual intercourse, homosexual

relations, and intercourse with animals of other species. The range of frequency of orgasm is very wide, from something over 30 orgasms per week, over several decades, to only one in thirty years. Among males under 31 years of age, average frequencies as low as once in two weeks, or lower, occur in only 11.2 per cent, average frequencies as high as once a day or more occur in 11.6 per cent. Age is the principal factor determining frequency of orgasm, the maximum occurring in the teens. There is a gradual but steady decline, thereafter, (practically a straight-line graph) approaching zero in extreme old age.

Marital intercourse provides a large proportion (about 85%) of the sexual experiences of those who are married. In single males orgasm occurs only about one third less frequently than in married males of the same age.

In a discussion of the social and legal implications of sex laws and mores and their enforcement, the authors state (p. 392), "85 per cent of the total male population has pre-marital intercourse, 59 per cent has some experience in mouth-genital contacts, nearly 70 per cent has relations with prostitutes, something between 30 and 45 per cent has extramarital intercourse, 37 per cent has some homosexual experience, 17 per cent of the farm boys has animal intercourse. All of these and still other types of sexual behavior are illicit activities, each performance of which is punishable as a crime under the law. The persons involved in these activities, taken as a whole, constitute more than 95 per cent of the total male population."

The percentage figures in the preceding paragraph refer to the incidence of the behavior in question, that is the proportion of males in whom the particular behavior has occurred at least once. The authors also give *frequency* data. In fact they provide, in a chapter of 54 pages, an elaborate series of "clinical tables," indicating the incidence and frequency of the different sexual outlets in various categories of age, education, and marital status. These tables are designed to facilitate the comparison of an individual man's sexual behavior with the pattern of the particular social group to which he belongs.

In a detailed consideration of the very diversified patterns of sexual experience Kinsey's investigations disclose a very considerable stability in the pattern of the individual male, and a considerable similarity in the patterns of those falling into the same social group. Educational level is the most significant single criterion, separating rather decisively the patterns of sexual activities of the groups whose education is destined to terminate at grammar school, high school or college levels. These patterns are not phases corresponding to the periods of schooling in the development of the individual, but appear to be pre-determined by basic differences in sex attitudes, set long before the actual top level of schooling has been reached. These findings raise interesting and important sociological questions as to the mode of transmission of sexual mores, on which the authors comment: "Children are the most frequent agents for the transmission of the sexual mores. . . . The so-called sex instruction which is given by parents and schools . . . has a minimum of any effect upon the development of patterns of sexual behavior, and, indeed, it may have no effect at all. . . . Exceedingly few males modify their attitudes on matters of sex or change their patterns of overt behavior in any fundamental way after their middle teens. . . . (Attitudes determining) patterns of behavior are estab-

lished long before the child is likely to have any comprehension of the nature of the legal formalization of our codes."

Early maturing males tend to become more active sexually than late-maturing males, and the higher rate of frequency of orgasm persists, whether married or unmarried, and whether the earlier outlet was in masturbation, permarital intercourse or some other sexual experiences. One finds in the data reported here nothing to justify the belief that sexual energy can be "conserved" or sublimated."

In the chapter on the stability of sexual patterns, one finds this illuminating statement: "Many persons, of course, believe that patterns of sexual behavior have changed considerably within the last generation or two. . . . One is inclined to suspect that the amazement of the older generation at the present day behavior is dependent, at least in part, upon the fact that the older generation knew very little about the behavior of the world in which it lived when it was young, and that it has only more recently become acquainted with the long-established facts of life." The data of the present study have been divided between younger and older males, differing by about 22 years. Comparison of their sexual behavior at like ages indicates that the sum total of the measurable effects of those twenty-two years have been "slight changes in attitude, some increase in frequency of masturbation among boys of the lower education levels, more frequent nocturnal emissions, increased frequency of pre-marital petting, earlier coitus for a portion of the male population, and the transference of a percentage of the pre-marital intercourse from prostitutes to girls who are not prostitutes."

To the clinical student of human nature, this book also brings a brilliant and wise discussion of interviewing. A brief quotation may serve to indicate something of the human quality which brought success in the actual carrying out of this investigation: "The many persons who have contributed to this study have done so voluntarily and with a full understanding of what we were trying to learn through our questioning. To have used any sort of devious device would have ruined the subject's confidence in everything we were doing. It has repeatedly been suggested that we try narcosynthesis, lie detectors, or other such means for testing the reliability of at least some of the answers of some of the subjects; but if we had coerced a single person by any such means, we would have lost our capacity to win things from anyone else. In any study which needs to secure quantities of data from human subjects, there is no way except to win their voluntary cooperation through the establishment of that intangible thing known as rapport."

J. C. W.

Textbook of the Ear, Nose and Throat. 2nd Edition. By FRANCIS L. LEDERER AND ABRAHAM R. HOLLENDER. Illus. 596 pp. \$7.00 F. A. Davis Company, Philadelphia, Pennsylvania, 1947.

This is the second edition of a textbook of otolaryngology which has been designed primarily, as the authors state in the preface to the first edition, for use by medical students. This concise book should be a great help to medical students who do not intend to specialize in nose and throat work. This book is not sufficiently detailed to be of great use to the student interning in otolaryngology, or

to the young specialist just going into practice. It is well arranged and has some excellent illustrations, especially those of laryngeal lesions as viewed by mirror examination.

The authors have written a type of book much needed by medical students to give them an introduction into the specialty of otorhinolaryngology.

J. E. B.

Textbook of General Surgery. 5th Edition. By WARREN H. COLE AND ROBERT ELMAN. Illus. 1160 pp. \$11.00. D. Appleton-Century Company, Inc. New York, New York, 1947.

The textbook of surgery written by Cole and Elman, and published by D. Appleton-Century Company, has just been revised in its fifth edition. The revision has apparently been thorough and incorporated the great advances made in surgery during the war with their application to civilian surgery. There is also a chapter on military surgery and another on chemical warfare, neither of which the present writer has seen included previously in other texts. This edition appears to be the most up to date publication now available as a textbook of surgery.

R. T. S.

Textbook of the Nervous System—A Foundation for Clinical Neurology. By H. CHANDLER ELLIOTT. Illus. 384 pp. \$8.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

The ambitious nature of the task undertaken in this small textbook is indicated by its title. The author attempts to present the important anatomical relationships and functions of the human nervous system in a diagrammatic fashion. He begins with a "bold but simple sketch" of what he terms the basic nervous system and fills in the details in later chapters. His object throughout is to correlate structure and function and to indicate the importance of such in clinical neurology.

The book succeeds in presenting the basic anatomical material in a clear and readily assimilable fashion. The first few chapters serve as an admirable introduction to the field. The illustrations are well done. Unfortunately, limitation of space leaves gaps in important details, which will need filling. The treatment of function leaves much more to be desired, and some aspects, such as those of cerebellar and motor systems, are inadequate. To the clinician, moreover, innumerable interjections of questionable or incorrect material dealing with points in pathology, diagnosis, and treatment, detract considerably from the value of the book.

Textbook of Clinical Neurology. 6th Edition. By ISRAEL S. WECHSLER. Illus. 829 pp. \$8.50. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

The chapter on psychological diagnosis has been rewritten and there have been other changes in this edition. It is likely that clinicians will hold it in the same regard as they have its forerunners.

BOOKS RECEIVED FOR REVIEW

- Brief Psychotherapy.* By BERTRAND S. FROHMAN. 265 pp. \$4.00. *Lea and Febiger, Philadelphia, Pennsylvania, 1948.*
- Conference on Liver Injury.* 127 pp. \$2.25. *Josiah Macy, Jr. Foundation, New York, New York, 1948.*
- Factors Regulating Blood Pressure.* Edited by B. W. ZWEIFACH and EPHRAIM SHORR. 175 pp. \$1.90. *Josiah Macy, Jr. Foundation, New York, New York, 1948.*
- Lehrbuch der Inneren Sekretion.* By F. VERZAR. Illus. 609 pp. *Verlag Ars Medici Ltidin A. G., Liestal, 1948.*
- Liver Injury.* Edited by F. W. HOFFBAUER. 74 pp. \$2.00. *Josiah Macy, Jr. Foundation, New York, New York, 1948.*
- Operative Gynecology, 6th Edition.* By HARRY S. CROSSEN and ROBERT J. CROSSEN. Illus. 999 pp. \$15.00. *C. V. Mosby Company, St. Louis, Missouri, 1948.*
- Oxford Loose-Leaf Medicine.* Edited by DR. HENRY A. CRISTIAN. 16 Chapters. *Oxford University Press, New York, New York, 1947.*
- Textbook of Surgery for Nurses.* By EDWARD S. STAFFORD and DORIS DILLER. Illus. 577 pp. \$3.25. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*
- An Outline for Teachers for use with A Textbook of Surgery for Nurses.* By EDWARD S. STAFFORD and DORIS DILLER. 65 pp. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*
- Treatment in General Practice, 6th Edition.* By HARRY BECKMAN. 1129 pp. \$11.50. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1948.*
- Tuberculosis.* By FRANCIS MARION POTTENGER. Illus. 597 pp. \$12.00. *C. V. Mosby Company, St. Louis, Missouri, 1948.*

VIRUSES AND VIRUS-LIKE AGENTS AS CAUSES OF CANCER

A BRIEF RECOUNTING AND REFLECTION^{1, 2}

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During the past dozen years we have all witnessed the impact upon medicine of what Sir Henry Dale has called the second era of research in chemotherapy, as manifested practically in the unparalleled usefulness of the sulfonamides, penicillin, and the newer antimicrobial drugs, and theoretically in the impetus which their discovery has given to new thought and experimentation on the metabolism of parasitic cells, the mechanisms of drugfastness, and the general principles of chemotherapy. Great as these achievements are, they were exceeded, in my opinion, by the attainments of bacteriology in the final quarter of the 19th century. For in that remarkable efflorescence, the genius of Pasteur, the logic of Koch, and the percipience of Lister permeated medicine with incredible rapidity, the immediate consequence being that a host of diseases which had puzzled and plagued mankind for centuries abruptly yielded their causes, and often their cures in addition, as fruits of the new science, while a vastly more important outcome was the fact that man had at long last identified and begun to study the bacterial creatures with which he lives. It was inevitable that these developments should influence thought about cancer.

For cancer was then what tuberculosis, typhoid fever, and anthrax had so lately been—baffling to the mind as well as sinister to the body, amongst the worst of man's afflictions however judged. True, its natural history had been half-disclosed, as much by the clinical and

¹The Alpha Kappa Kappa Lecture given in Hurd Memorial Hall, The Johns Hopkins Hospital, November 18, 1947.

²Recent investigations in the author's laboratory on viruses and virus-like agents in relation to tumors have been supported by the Jane Coffin Childs Memorial Fund for Medical Research.

anatomical studies of Morgagni, Pott, Bichat, John Hunter, Laennec, and their like, as by the histological observations of Johannes Müller, Remak, Virchow, Cohnheim, Ribbert, Waldeyer, Hansemann, Thiersh, and Pianese, to name but a few of that army of workers who had set about exploring the human body cell by cell as soon as the compound microscope had become available. But only the wooliest notions existed then as to the possible causes of cancer, and nothing was known about the disease that weighed very heavily against the supposition that it might be caused by a pathogenic bacterium. Indeed the erroneous but prevailing doctrine of Broussais and Virchow—that cancer is but a form of inflammation—must have made the possibility seem quite near. In any event, many workers acted on it, and some spent their scientific lives isolating from cancer after cancer bacteria of many and diverse sorts, trying with these to reproduce the tumors under controlled conditions in experimental animals. Alas, their results were uniformly negative or unconvincing, and eventually they all had to accept it as a fact that causative bacteria cannot be grown from cancers. And eventually interest waned in the old superstitions about “cancer-houses”, “cancer-streets”, and “cancer-villages”, which had made it seem that cancer was catching and which had got their start from a series of inexact observations and uncritical inferences that must have made Claude Bernard turn his face, the more so since they had originated in France. Things looked gloomy indeed for the hypothesis that cancers may be caused by extraneous pathogenic agents.

But then the filtrable viruses made a discreet entry onto this bustling bacteriological scene, first tobacco mosaic virus in 1892 and then that of hoof and mouth disease in 1898. Reason enough, it seemed to many, and notably to a bold and imaginative Frenchman named Borrel, for further thought about the parasitic hypothesis of cancer causation. For these new agents produced disease even though they could not be seen with the microscope or grown on lifeless media. Did they cause cancer? Far from overlooking the new possibility, Borrel rushed headlong to meet it. “The problem of cancer causation had taken entire possession of his eager, inquiring mind,”—as Oberling tells us in *The Riddle of Cancer*, “and he had examined tumors by the hundreds and from all possible sources, animals, and man, for a specific

microbe. All in vain. Too critical to let himself be carried away with other bacteriologists by inadequate proofs, or to persevere in a line of investigation now so obviously destined to lead nowhere, he conceived the notion that he might be facing the same difficulties that had baffled Pasteur in his search for the cause of rabies. A virus seemed the only plausible solution." In a remarkably percipient essay published in 1903, Borrel pointed to the proliferative character of the initial cellular changes in small pox and molluscum contagiosum, and compared these with cancer (9). His thought has elicited sharp criticism, but the facts upon which it was based have proved sound, and from much subsequent work, notably that of Philibert (43), Rivers (45), and Shope (54, 55), the principle has emerged that viruses can make cells grow as well as cause their death. Upon this principle and another since apprehended—namely that viruses can lie latent in tissues, producing lesions only when provoked into pathogenicity by one or another of a variety of stimuli—recent protagonists of the view that viruses cause cancer have mainly built their arguments (3, 49).

OLDER WORK ON FILTRABLE AGENTS AND TUMORS

It is one thing, however, to recognize the principle that a few viruses can bring about more or less transitory proliferation—the majority being swiftly lethal to their cellular hosts—and quite another thing to ask whether viruses actually cause cancer. Some of the facts that bear upon this question may be cited briefly and chronologically as follows.

In 1902 the observation was reported that a filtrable virus is responsible for fowl pox, and subsequent events have shown that this interesting disease process deserves a more conspicuous place in the history of cancer research than the one into which it has fallen (22). Bollinger, one of the first to study fowl pox histologically, classified it as a tumor, even though it was manifestly infectious; he named it epithelioma contagiosum in 1873 (8), and others since have noted its resemblance to molluscum contagiosum of human beings. Today, however, fowl pox is not thought of as a neoplasm; for it is essentially a self-limited process, necrosis eventually following proliferation in the diseased cells, which mummify and are cast away in due course with complete healing underneath if the host has not died meanwhile from

an associated sepsis.³ Its importance in the history of cancer research lies in the fact that it was the first tumor-like process that was proved to be caused by a filtrable virus; as such it inevitably influenced the subsequent study of tumors in fowls, which we may next consider.

In 1903, Ellerman and Bang reported that they had been able to transmit leukemias and lymphomas of chickens by means of filtrable agents, and their findings were immediately confirmed by Hirschfeld and Jacoby, and later by Schmeisser (17). The fact that the blood stream was conspicuously invaded in these disease processes coupled with the fact that filtrable agents had been found responsible for them, seemed sufficient reason at the time to rule them out as tumors, and many years elapsed before their neoplastic character became generally recognized (18).

In 1911, Peyton Rous made the significant discovery that a sarcoma of fowls, which he had previously identified as a neoplasm by careful biological and morphological studies, was transmissible by means of a filtrable agent (48). He and his associates soon determined that a number of other sarcomas of fowls would likewise yield filtrable agents of a similar sort, and that each agent would elicit a growth exactly like that from which it had come if brought into contact with normal fowl mesenchyme under appropriate experimental conditions. The findings have been confirmed in laboratories all over the world, and perhaps extended here and there (13, 19). It has become plain that they stand apart and comprise the bulk if not the sum of what we know today about cellular factors that actually cause cancer.

MORE RECENT WORK ON VIRUSES IN RELATION TO CANCER

The findings just mentioned long stood alone as providing evidence that filtrable agents may be present in cancer cells and responsible for their malignancy, for during more than 20 years the repeated attempts of workers everywhere to extract causative viruses from tumors of many sorts in mammals and other species all proved unsuccessful. Lately, however, the picture has acquired more detail.

³ It is interesting to note in passing that within the past few years the disease, occurring epidemically in wild sooty grouse, has been mistaken for cancer—vide, *Journal of Wildlife Management*, 4: 311–312, 1940.

In 1933, Shope made the discovery that the cutaneous papillomas of Western cottontail rabbits are caused by a filtrable virus that will reproduce the growths when rubbed into the scarified skin of other rabbit species, both domestic and wild (55). Rous and Beard next showed that the virus-induced papillomas, though benign, possess certain of the attributes of neoplasms (50), and that the growths in domestic rabbits, after remaining harmless for periods of several months to a year or more, almost regularly become squamous cell carcinomas that metastasize and kill their hosts (51). Rous and Kidd then found that the papilloma virus will call forth in great profusion a variety of carcinomas, as well as papillomas, when brought into contact with tarred epidermis (53), and Rous and Friedewald later showed that carcinomas appear within a few weeks in papillomas exposed to methylcholanthrene more or less constantly from the time of their inception (52). The relationship, however, between the virus, which initiates the benign papillomas, and the cancers, which come later, has proved exceedingly complicated. For while readily recoverable in large amounts from the natural and experimental papillomas of wild cottontail rabbits, the virus is only occasionally demonstrable, and then in comparatively small amounts, in extracts of the papillomas produced with it in domestic rabbits, and it could not be extracted at all from the cancers, as much work showed (56, 27, 21). In this unsatisfactory position, with the essential facts unlearned about the state and functions of the virus in the papilloma and carcinoma cells of wild and domestic rabbits (33), the problem rested for quite some time, though indirect evidence, procured by means of serological and immunological experiments, made it plain that the papilloma virus, in masked or altered form, persisted in the cells of two transplanted cancers that had originated in virus-induced papillomas, and increased in amount as the cancer cells proliferated (34, 29). The inference seemed warranted that the virus, obviously responsible for the initial proliferation of the papilloma cells, likewise impelled the cancer cells with which it remained associated. But quite recently an extraordinary fact has emerged with respect to the relationship between the virus and the cells of the V2 carcinoma (this being the second of the two transplanted cancers mentioned above, the first having been lost early in the course

of transplantation). For having persisted in the V2 carcinoma cells for more than five years and throughout the first 25 successive tumor generations, the antigenic virus has now, after a further interval of five years, disappeared from them; or at any rate it is no longer disclosed in them by serological experiments of the sort that regularly accomplished this before (57). Can it be that the papilloma virus went along with the carcinoma cells merely for the ride, so to speak, playing no very essential part in their malignant activities during its long association with them? This seems not unlikely, for the V2 carcinoma cells are now, in the absence of the virus, precisely what they were in its presence, so far as can be told from their appearance and behavior, while the fact is well known that non-neoplastic viruses may thus ride along in tumors (37, 47, 29, 60). Wholly apart from this new and unexpected development, I have elsewhere indicated reasons for supposing that a constituent of the V2 carcinoma cells other than the papilloma virus may be responsible for their continuing malignancy and may indeed have initiated their malignant transformation (32). More will be said further on about this.

The observations of Lucké, on the etiology of certain renal adenocarcinomas in frogs, broaden the base of our theme. In 1934, he noted that acidophilic intranuclear inclusions are conspicuous in the cells of these tumors and similar to those previously found by Dorothy Russell in the elements of gliomas in human beings (39). Lucké later showed that the frog carcinomas can be produced experimentally by means of a filtrable agent extractable from them (39); the causative agent, however, acts only sporadically and after a long incubation period, and its properties have not been fully defined.

The foregoing review of recent work on viruses in relation to tumors could easily be enlarged by references to scores of additional papers, but it seems doubtful that it would be notably enriched thereby. For the bulk of the papers that might be cited report at most but microscopic extensions of principles already largely understood from previous work, while some are filled with fallacies, and others lack strategic orientation and perspective. By no means are virus-workers immune from Woglom's pointed dictum (64): "—Few investigators seem to realize that cancer research is a discipline requiring some apprentice-

ship, and that not everyone with an inoculating needle and a dozen white mice can plunge in and emerge with a discovery." The recent claims of Taylor, that filtrable cancer-producing agents can be recovered from mammalian tumors grown in developing chick embryos, would seem to merit our attention only in relation to the reports from other laboratories that the essential observations are unverifiable (61). And all of the extensive work with the Shope fibroma may logically be omitted from consideration; for although knowledge about this virus-induced process has added substantially to the principle already referred to that viruses can make cells proliferate, and although the lesion is "tumor-like," as Shope said (54), it is not neoplastic, the nodules produced by the virus being invariably self-limited and composed for the most part of exudative fluid and "sick" fibrocytes that eventually die and are resorbed.

VIRUS-LIKE AGENTS AND CANCER CAUSATION

To think about viruses in relation to cancer would hardly be feasible without taking count to some extent of the vexed problem of what viruses are. Are they parasites, once free-living but now dependent on their cellular hosts—the simplified products of a retrograde evolution, as Green (24), Laidlaw (36), and Burnet (11) have supposed? Or are they non-living protein molecules, perhaps derived initially from the host's own protoplasm, as Stanley's work may indicate (59)? Or may they be microbial midguts of diverse kinds, as a class occupying and perhaps bridging the twilight zone between the living and the dead in nature, as Rivers has imagined (46)? It is not profitable to our theme to do more now than ask these questions, yet it may be helpful to have them before us as we consider certain other agents that may be concerned in the causation of cancer.

The first of the agents that I shall call virus-like and here discuss is that implicated in the causation of cancer of the breast in mice. It has long been known that the females of some breeds of mice are prone to develop cancer of the breast, while those of other lines are virtually free from the disease; and it was of course assumed that this predisposition was inherited by way of the genes until facts proved it otherwise. In 1933 and 1934, it became clear from experiments done independently by the Staff of the Jackson Memorial Laboratory in this country (38)

and by Korteweg in Holland (35) that the tendency to mammary carcinoma is passed along only by way of the mother: if she be of high-cancer stock, her progeny develop breast cancers largely irrespective of their paternal heritage, whereas if her high-cancer strain brother be mated with a low-cancer female, their progeny are usually devoid of the disease. Bittner then showed, in 1936, by means of foster-nursing experiments that this "maternal influence"—obviously "extra-chromosomal"—is carried in the mother's milk (7), and much study has since been made of the distribution, properties, and potentialities of the "milk factor" (2). It manifestly passes from teat to mouth during suckling and increases in amount during its sojourn in successive hosts; and in concert with certain hormonal and constitutional influences, it obviously plays a crucial part in the etiology of one type of mammary adenocarcinoma. Yet, as much work has shown, it is harmless to mice of many strains and to males and castrated females of susceptible lines unless hormonal stimulation is given artificially to them during long periods of time. It seems especially noteworthy that the milk factor has not caused cancer directly under any of the experimental conditions devised thus far. As I have pointed out elsewhere (32), we remain ignorant of whatever part it may play in producing the malignant change that finally comes about in the benign mammary adenomas in mice susceptible to breast cancer, and no evidence exists that it is essential to the continued proliferation of the malignant cells. Furthermore, its physical, chemical, and antigenic properties as defined thus far do not suffice to distinguish it as a virus, the sedimentable constituents of normal tissue cells being comparable to it in the properties deemed significant. Virus-like indeed the milk factor is, when considered broadly, but the fact deserves reiteration that its precise position in the hierarchy of things and its actual part in the pathogenesis of breast cancer in mice remain yet to be ascertained. It may well be a carcinogenic agent—in the sense that under highly specific circumstances it brings about intracellular conditions favorable to the onset of malignancy—but in the end, like the Shope papilloma virus, it may turn out to be an epiphenomenon so far as the continued causation of cancer is concerned.

Finally, we may consider briefly, in relation to the problem of malignancy, two virus-like agents that can be identified by serological means

in tumors. Some years ago, for reasons which I shall not here relate, I was led to ask whether the methods of serology might prove useful as a means of demonstrating in cancer cells the substances that make them such (28). From an extensive series of investigations along these lines, it has become plain that the cells of the Brown-Pearce rabbit carcinoma, a typical transplantable cancer of unknown cause, will regularly yield a distinctive substance which is identifiable by serological means. This material has not been detectable in extracts of other rabbit tissues, either normal or neoplastic; in a number of experiments it did not elicit tumors when brought into contact with normal and experimentally altered rabbit tissues, yet upon analysis it proved to have certain physical and chemical properties remarkably similar to those of the viruses—notably a large particle size and weight as determined by ultrafiltration and ultracentrifugation—and other properties which suggest that it is probably a protein and perhaps a ribose nucleoprotein (30). The subsequent studies of Claude and others have shown, however, that these physical and chemical properties are likewise shared by particulate components of many normal tissues, though it is clear that the specific constituent of Brown-Pearce tumor cells can be readily distinguished from the particulate components of normal rabbit tissues by serological means (30). Like the filtrable agents responsible for fowl sarcomas, the distinctive constituent of the Brown-Pearce carcinoma cell seems to be associated with, or to concur with, constituents of its malignant host cells that are similar in chemical and serological properties to the cytoplasmic microsomes of normal tissue cells as defined by Claude (14, 30, 31, 32)—an association that may have meaning in view of the time-honored implication that the formed constituents of cytoplasm play important parts in the activities of cells, as for example in determining differentiation and in mediating synthetic activities as well perhaps as in providing active centers for energy metabolism. Recent observations have provided evidence that the distinctive constituent plays an essential part in the proliferative activities of Brown-Pearce carcinoma cells, for it has been shown that the antibody that reacts specifically with the distinctive constituent suppresses the growth of living Brown-Pearce carcinoma cells under a variety of experimental conditions (31). In other serological investigations, facts have been uncovered which indicate that another substance, similar to

that of the Brown-Pearce carcinoma cell yet readily distinguishable from it serologically, is present in the cells of the V2 carcinoma, already mentioned, and absent from benign papilloma cells of the type from which they sprang. The findings considered together have led me recently to inquire (32) whether distinctive constituents such as those associated with Brown-Pearce and V2 carcinoma cells may not be present in the generality of cancer cells and responsible for their continuing malignancy, each type of neoplastic cell having perhaps a special kind of constituent which, by its intracellular activities, determines the particular misbehavior of the cell (autonomy), and which, by crowding out or overgrowing or suppressing the activities of the cell's normal constituents, brings about a degradation of its normal form and functions (anaplasia).

* * *

In medical science, as in fishing, it may be important to have a good backcast if one's forecast isn't to be a mess. Hence I have tried in this small essay to provide perspective as well as intellectual provender for those who may wish to think particularly about the actuating causes of cancer. Although pathetically small in number and permeated through and through by uncertainties, the examples herein cited comprise the essence of what we know today about the intrinsic factors that make cancer cells what they are.⁴

Recently it has become fashionable to suppose that further advances in knowledge about cancer will come largely, if not entirely, from new discoveries in one or another of the fields of intracellular physiology, chief encouragement being derived from prospects in the study of growth as a phenomenon. As a result, a whole host of new workers has begun to think about cancer, and during the past few years its

⁴No consideration need be given to the so-called carcinogenic agents—tar, polycyclic hydrocarbons, x-rays, sunlight, arsenic, butter-yellow, etc.—in this relation. For while these agents may initiate cancer, it is well known that they like certain of the agents herein described, play no essential part in the continuing and malignant activities of cancer cells. Nor should we be deterred even momentarily by the resounding phrase *somatic mutation* in explanation of cancer, the term representing at present either a supposition inherently untestable by Mendelian methods and one against which formidable theoretical objections can be raised, or a pretentious and misleading label for the unknown, as I have pointed out in another place (32).

causation has been explained theoretically by analogies drawn from a number of rather distant sources. For example, from the hypothesis that plasmagenes are influential in determining the structure and behavior of tissue cells (plasmagenes being hypothetical cytoplasmic entities presumably analogous to nuclear genes, and, like the latter, possessing molecular dimensions as yet undefined), a number of authors have advanced the supposition that aberrant forms of these conjectural entities may be responsible for malignancy (15, 25), while from the conception that mutant plastids cause variegational diseases and viroses in plants, and from the supposition that all tissue cells harbor ultra-microscopic symbionts analogous to the intracellular "bacteroids" of cockroaches, other authors in turn (65, 1) have assumed that anomalous forms of those entities may cause cancer, and still other essayists have put forth related formulations having points of orientation somewhat less discernible. It is indeed interesting that a number of scientists working in different parts of the world on widely various aspects of cellular biology should more or less simultaneously synthesize the view that variations in cytoplasmic entities may be responsible for the permanent, irreversible, and heritable alteration in tissue cells that constitutes cancer. Yet too much emphasis cannot be laid on the point that all of this is mere hypothesis, now jerry-built upon other hypotheses, and comparable perhaps to the first formulations of the somatic mutation hypothesis of cancer causation some thirty years ago; hypothesis it will remain unless altered cellular constituents can be demonstrated in malignant cells and shown to be responsible for their malfunction.

The broadening of the attack on cancer, however, is of course all to the good, for in a problem so vast and intricate who can tell whence the next advance may come? And who can assay the benefits to it of the substantial achievements of cytology and genetics, old and new (63, 10, 41, 26, 62), and of the factual contributions and important implications provided by recent work on a wide variety of cells (6, 58, 44, 12, 5, 23, 4, 16)? And what mightn't it profit the oncologist to know in detail all of what remains yet to be learned about growth and differentiation in normal cells—to comprehend, for example, the minutiae of mitosis, the vital doings of cytoplasmic and nuclear nucleoproteins, and the riddles of protein synthesis, of energy metabolism, and of specialized

cellular functions, not to mention the scores of related problems that immediately come to mind. Yet MacCallum was wise, it seems to me, in putting the section on tumors at the end of his *Textbook of Pathology* (40), along with this statement: "—We are quite familiar with the effects of every sort of injurious influence in disturbing temporarily the normal growth of tissue, and can formulate general laws for these effects which are found to be always respected. But tumors do not respect them at all. For that reason I have written of tumor growth separately from all the rest of pathology as a thing apart, not to be dealt with according to the laws of reaction of normal tissues." Thinking along the same lines, Foulds, an experimental pathologist, in a detailed and closely-reasoned histological and biological analysis of tumors (20), has written: "It is far from certain that complete knowledge of the forces which control the development and maintenance of normal structure would solve the problems of cancer." Should the point of view be submerged by current coordinations of cancer research?

In striking contrast to all of the factors that work for the good of cells, the viruses are predatory villains, invisible and notoriously subversive, yet dramatic and conspicuous in their effects, their activities as a rule bringing sickness and death to their cellular hosts. The sum of what has been learned about them and about their effects on cells, however, has not been without profit to cellular biology; in quantity and in precision it compares not unfavorably with what can now be marshalled about the nature and effects of other agents—cellular constituents, hormones, organisers, inducers, for example—that comprise and influence the structure and function of normal cells. It is quite conceivable that the viruses may prove increasingly useful as reagents for the study of intracellular processes, both normal and abnormal. Perhaps, indeed, the dim and uncharted lane between intracellular pathology and intracellular physiology can with profit be trod in both directions, becoming in due course a travelled and well-marked highway. Even so, it may prove important to encourage those who seek within the cancer cell for the cause of its enduring malignancy; for therein it surely lurks, and perchance also the means for its control, whereas it may be latent or submerged in non-neoplastic cells, or even absent from them. For all, however, who would learn the ways of cells, the examples herein described, of viruses and virus-like agents in relation to their malignant hosts, may serve as useful guides.

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THERAPEUTIC CONFERENCE THE TREATMENT OF EPILEPSY

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Dr. A. McGehee Harvey: The subject for discussion today is the management of patients with epilepsy. Until recent years, most of our knowledge developed in a rather empirical fashion. Investigative techniques now available have resulted in an increased interest in this symptom complex, and facts which have a sound experimental background are rapidly accumulating. In view of the intense clinical and experimental activity now going on in this field, it seems appropriate to bring together the pharmacologist, the neurophysiologist, the neurologist, the neurosurgeon, and the psychiatrist in a discussion of the present state of our knowledge and the future trends in research.

The first speaker will be Dr. Butler, who will tell us about anti-convulsive drugs.

Dr. Thomas C. Butler: This discussion will be concerned with only one aspect of the management of epilepsy, the suppression of the seizures by means of drugs. It will be devoted principally to the problems of detecting antiepileptic activity in the laboratory.

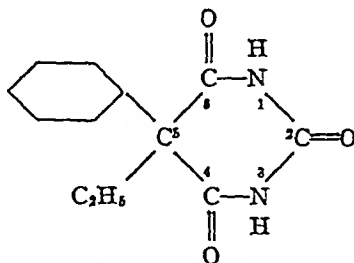
The bromides and phenobarbital, the principal drugs in use ten years ago, had both found their way into the clinical treatment of epilepsy without the benefit of any preliminary laboratory investigation directed toward a search for that particular type of activity. Potassium bromide, the first really effective agent against epilepsy, was introduced in 1857, and it owed its trial to theoretical reasoning that proved to be fallacious. Sir Charles Locock had read the report of a German who had taken potassium bromide himself and had noted as one of the effects of the drug the development of sexual impotence. Locock, thinking that the drug might have some specific effect on sexual functions, tried it on some female patients who had attacks of what he termed "hysterical epilepsy" in connection with the menses. He found the drug efficacious and later extended its use to other types of epilepsy. Even though the reasoning that led to the trial of potassium bromide may have been scarcely more valid than that which had suggested the use of hundreds of other remedies during the preceding centuries, potassium bromide happened to differ from its predecessors in being a really effective antiepileptic agent, and it was not long before it was so recognized.

The effectiveness of phenobarbital was disclosed as the result of clinical trial of every hypnotic agent that became available. After the first report of its use in epilepsy in 1912, it gradually began to be realized that phenobarbital, in comparison with other hypnotics even in the barbituric acid group, had antiepileptic activity out of proportion to its hypnotic activity. The accumulating experience

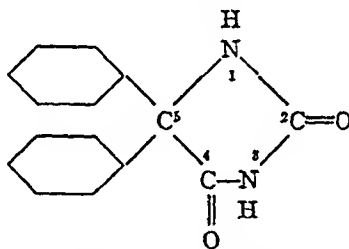
with hypnotic agents in epilepsy was giving increasing evidence that antiepileptic activity was not a simple manifestation of general anesthetic activity.

The drugs of greatest interest that have been introduced during the last few years have received their clinical trial as a result of laboratory tests designed to uncover antiepileptic activity. This is not to say that the tests we have are known to be completely reliable guides. Nevertheless, whether for entirely valid reasons

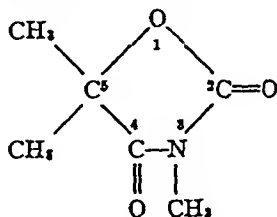
FIG. 1. STRUCTURAL FORMULAS OF SEVERAL ANTICONVULSANT DRUGS



5-ethyl-5-phenyl barbituric acid, or phenobarbital



5,5-diphenylhydantoin, or dilantin



3,5,5-trimethyloxazolidine-2,4-dione, or trimethadione ("tridione")

or not, they have led to the trial of drugs that have proved valuable therapeutically and which promise to give a better insight into the nature of antiepileptic activity.

The study of the so-called experimental epilepsies is by no means new. There are many ways of causing animals to undergo convulsive seizures which bear more or less resemblance to the attacks in the epileptic patient. On account of the ease of producing them, the most thoroughly studied laboratory seizures are those produced by convulsant poisons and those produced by electrical stimulation of the brain.

Fritsch and Hitzig, who in 1870 first discovered the effects of electrical stimulation of the motor cortex, also described the convulsions that could be produced by strong stimulation. They were able to duplicate the development of a typical Jacksonian seizure, and so confirmed Hughlings Jackson's earlier speculations as to the nature of these attacks. It was immediately realized that this means of producing epileptiform seizures furnished an opportunity of testing drugs to be used in human therapy. Hitzig studied the effects of ether and morphine on cortical excitability. The same method was used by a few other investigators of that period to study the effects of some of the anesthetics and hypnotics then in use. However, the number of promising drugs available at that time was so small that this represented no progress in the search for antiepileptic drugs.

At the beginning of this century groups in France and Switzerland were experimenting on the convulsive effects of the passage of interrupted direct current or of alternating current through the intact head. This method was used as early as 1904 for testing several well known drugs, but the first application to the testing of a large series of compounds, with the view of uncovering antiepileptic activity, was the work of Merritt and Putnam in 1938. Merritt and Putnam used cats, with one electrode in the mouth and one on the occiput. They found that the familiar drugs produced effects on the convulsive threshold correlating with their known effects in human epilepsy. A number of compounds, not previously tested as antiepileptics, were found to be active in raising the threshold.

As a result of this experimental work, one of these compounds has had a very thorough clinical investigation. This is 5,5-diphenylhydantoin. Hydantoin is a compound structurally related to barbituric acid, and the 5,5-disubstituted hydantoins resemble the corresponding barbituric acids rather closely in their pharmacological properties. This particular compound, like a number of other hydantoins and barbituric acids, does not produce smooth anesthesia in animals. It produces a picture of combined excitation and depression, and in low doses actually appears purely convulsant. It affords no protection against convulsant drugs such as metrazol. According to the ideas then prevalent as to the nature of antiepileptic drugs, diphenylhydantoin is a drug that would not have been expected to be active and doubtless would never have had a clinical trial had it not been tested in this way. The tests in man soon showed that the drug was, in fact, a highly effective agent in suppressing grand mal seizures, that it produced less hypnosis than phenobarbital, and that it differed qualitatively from phenobarbital in its action in that it was effective in types of epilepsy where phenobarbital was ineffective.

The success of the method of Merritt and Putnam in disclosing such a valuable and interesting drug as diphenylhydantoin naturally has led to the very widespread testing of drugs by similar procedures. Comparison of the results of various investigators leaves one with a picture of confusion and conflict. While some are in essential agreement with Merritt and Putnam, others find that diphenylhydantoin does not raise the convulsive threshold at all, or has less effect than phenobarbital. This conflict apparently stems from the fact that almost every worker has used different placements of electrodes, different durations of shock, different

types of current, and, probably most important, different species of animals. There is some reason to believe that in different species, or with the passage of current through different paths, the convulsive discharges may arise in different foci. Experiments have been reported which suggest that diphenylhydantoin influences only those convulsions arising in the cortex. Further efforts to elucidate the site and nature of the action of known antiepileptics would seem profitable in order to put the testing of new agents on a sounder basis.

During the last four years, considerable study has been devoted to a drug which has been found to differ from the older antiepileptic agents both in laboratory and clinical tests. This drug is trimethadione. It is a derivative of oxazolidinedione, a compound which also resembles barbituric acid in structure. Derivatives of oxazolidinedione in which the 5-position is substituted with two alkyl groups have hypnotic activity resembling that of the corresponding barbituric acids. This particular compound is hypnotic, but one of the least active hypnotics among the oxazolidinediones that have been studied. It has been found active in raising the convulsive threshold in electroshock, even by those techniques that fail to show an action of diphenylhydantoin. Unlike diphenylhydantoin, but like phenobarbital and other barbituric acids, it is an effective antagonist of metrazol and certain other convulsant drugs. The surprising finding on clinical trial was that the drug, while failing to suppress grand mal attacks, was quite effective in petit mal.

From the knowledge now at hand, it would be difficult to make any final appraisal of the value of laboratory methods at our disposal for studying antiepileptic activity. By electrical stimulation it is possible to duplicate quite closely those seizures arising in the motor cortex, not in respect to the initiating cause but in respect to the spread of the neuronal discharge. Because of the difference in the initiating processes, it is not *a priori* obvious that all drugs will behave similarly in the two conditions. However, the electrically produced convulsion, at least in some animal species and in the hands of some investigators, has shown drug response in very good correlation with grand mal epilepsy in man. Those clinical seizures arising from discharge in other parts of the brain than the motor cortex are more difficult to reproduce and study in the laboratory. Trimethadione has been responsible for a revival of interest in the antagonism to convulsant drugs. The relationship of activity of this type to activity in petit mal epilepsy is by no means clear. The metrazol convulsion, although obviously not duplicating at all closely a petit mal seizure, may have some points of similarity. Of what value it will be in uncovering activity against petit mal is problematical.

These three drugs, phenobarbital, diphenylhydantoin, and trimethadione, have had sufficiently thorough clinical investigation to establish that they have qualitatively different types of action. It is to be hoped that intensive laboratory study of these drugs may clarify the basis of their differences and indicate the means by which superior drugs may be searched for in the future. However, it is obviously unsafe to base any very far-reaching generalizations on experience with such a small number of drugs. Any tentative conclusions must be tested eventually by careful clinical studies of a much larger number of appropriately selected drugs.

It may be worthwhile to speculate briefly as to the future in the field of anti-epileptic drugs. There is certainly reason to desire better drugs than those we have now. Diphenylhydantoin and trimethadione have brought with them therapeutic benefits not furnished by phenobarbital, but they have also brought toxic effects not produced by phenobarbital. There is no reason to believe that these particular compounds are the best members of their own chemical types, or that antiepileptic activity is confined to structures closely resembling these. So it does not seem unreasonable to hope that the present drugs will be improved upon, certainly in respect to toxicity if not in respect to effectiveness.

Dr. Harvey: Dr. Magladery will now take up the management of epilepsy from the viewpoint of the clinician.

Dr. John Magladery: This morning we are going to discuss the management of the epileptic patient. It is a subject replete with many therapeutic facets, any one of which might be an ample topic for such a discussion. For obvious reasons, therefore, we shall attempt to keep this presentation on a very general level.

It is well to take stock before proceeding to a consideration of measures which may be adopted to combat or modify any pathological process or disease entity. One must be familiar with the natural course of the abnormality, recognize its more common manifestations, appreciate the significance (when present) of certain features, understand what assistance may be obtained from special investigations and, perhaps more important, realize the limitations of such. Finally, and this is especially true of today's subject, one must be well aware of the dual relationship of the abnormality to the patient as a human being—the emotional responses evoked by the disturbance, and the possible modifications of the abnormality by the emotional state of the individual.

It has become apparent that one must now limit the term epilepsy in some way. Clearly, there are many apparently normal individuals showing some degree of alteration in electro-encephalographic pattern, of an intermittent character, and that this "epileptic tendency", as it is sometimes called, shows strong hereditary features. Perhaps this represents a tendency on the part of such individuals to develop clinical seizures in the face of alterations in blood flow, cellular chemistry, intracranial pressure relationships, and so on, which would not lead to such per se in other people. The cause of such tendency is of course completely unknown and will presumably remain so until we have some knowledge of the basis of the different neural circuits contributing to normal rhythmic cortical activity, factors influencing facilitation and inhibition, suppression, and so on. For various reasons, however, and often for no apparent reason, these individuals may develop clinical seizures. This tendency to recurrent excessive discharges when it produces clinical attacks or fits, we term epilepsy, and we are thrust back on a clinical understanding of the features of this symptom.

Here it becomes difficult. The clinical aspects of epilepsy are so many and so diverse that any formulation based on symptoms is hardly feasible. There are

discrepancies in any rigid criteria even as to what constitutes a fit. Prolongations of aura or post-ictal states may even detract from the comparatively sound clinical guides of sudden onset, brief duration (a few minutes at the most), and recurring habit.

The possibility of establishing a working etiological classification of epilepsy seems more likely now than ever before. The roles of hypoglycemia in some seizures, overventilation in others, hydration, the influence of minor cortical structural changes, in addition to more gross abnormalities, have all reached established acceptance. This is so true that there seem reasonable grounds for the view that all epilepsies are really "*symptomatic*". Nevertheless, we are currently forced for tutorial reasons to confess our ignorance in the large majority of cases and lump them together as "*cryptogenic*", or of obscure origin. There is nothing distinctive or mysterious in the latter group to separate it from the symptomatic epilepsies—either may show any of the descriptive subgroups which have clinical similarities or are sometimes (as stressed by Gibbs and Lennox) accompanied by rather distinctive electro-encephalographic patterns: Jacksonian, grand mal, petit mal, psychomotor, akinetic, etc. I do not propose going into descriptions of these today. Our important problem in distinction *must be*—is this epilepsy initiated or favored by an infection, structural lesion, or metabolic change which lends itself to correction by present-day means or, on the other hand, can our therapy be directed solely to the modification of the underlying cerebral dysrhythmia and of the patient as a human being? In other words, is this symptomatic or cryptogenic epilepsy?

The differentiation, of course, devolves on an understanding of the clinical features of these various factors (and time does not permit discussion of these factors) but it can be furthered in certain ways. An important one lies in the age of the patient. Another lies in the attention to focal symptoms and signs—the latter absent, and the former uncommon, in cryptogenic epilepsy. Finally, further assistance can be obtained from special measures such as skull and chest x-rays, blood serological tests, urinalyses, and fasting blood sugars. Some groups would puncture and do encephalograms on all epileptics, and there is much to be said for that practice. This can be reserved, however, for those with other suggestions of intracranial space-occupying or focal disease *only* by accepting the responsibility of frequent examination of the patient. It must be remembered that in several large series, 40–50% of all patients with seizures secondary to tumors had them as initial symptoms. Electro-encephalograms used indiscriminately are of very limited value. Patients in the older age groups and those showing focal lesions are important in the search for focal or predominantly localized changes.

The plan of treatment of each epileptic patient must develop in an orderly fashion and be directed towards various factors involved. There is rarely any great need for immediate institution of therapy, and each case merits detailed consideration of these factors before and during treatment. It is not merely a

question of administering certain drugs in fixed dosages to patients, but rather of the institution of a studied long-term care of the individual.

Certain aspects of treatment associated with the various precipitating factors indicated above need only to be mentioned again—the operative treatment of foci associated with space-occupying lesions, trauma, vascular anomalies and the adequate treatment of syphilis—the management, medical or surgical, of hypoglycemia—and so on. Let us rather consider what measures we have for the prevention or modification of the epileptic attacks themselves, be they symptomatic or cryptogenic.

In the first place, attention must be turned to the patient himself. It is uncommon to find epilepsy first showing itself in the face of emotional difficulties or during the course of a psychogenic illness. Yet it is common experience that, once initiated, the frequency and continuance of attacks often depend on the emotional state of the patient. They are more frequent with insecurity and lack of purpose. In some cases, infrequent attacks may be completely controlled by good psychiatric management alone.

The converse is more strikingly evident. Every individual subject to repeated epileptic attacks faces a tremendous hurdle in his attempts at adjustment in society. If these have started early in life there is every possibility that he has been over-protected by his family; has been considered as different, at the very least, by his school fellows; has, in some cases, been deprived of schooling; has repeatedly been the centre of undue interest, either protective or horror-stricken. In adult life, these features are augmented by failure to obtain or hold employment, the barrier of marriage, and so on. It is not surprising, therefore, that personality changes in response to fear, frustration, repeated failure, are common amongst epileptics. Most observers agree that there is nothing specific about the pattern though numerous references to the so-called "epileptic personality" are to be found in the literature. Assistance must often be given then to the individual in his social adjustment, in rehabilitation measures, employment.

Deterioration in cryptogenic epilepsy is a vexing problem. It is unquestionably related in many cases to the psychogenic factors mentioned above; in others to the post-convulsive state associated with frequently-occurring seizures; in others to the too-enthusiastic use of medication. The correction of these influences often leads to a marked improvement. In a few cases unfortunately, and the explanation is not clear, deterioration is progressive and eventually may require institutional care.

It is necessary, for the protection of the patient and others, to place certain restrictions on his activities, on driving a motor car—working in various potentially dangerous occupations—and so on, at least until he has shown himself under perfect control for a long period. This is introduced at this point to stress the fact that these restrictions should be specific and minimal, and their imposition should be made with full cognizance of their emotional value to the patient.

We can pass quickly over general measures in the control of the epileptic at-

tacks. The work of McQuarrie, Temple Fay, and others has emphasized the relation of fluid intake to the frequency of seizures. Marked dehydrative measures do reduce their incidence in large series; excessive fluid intakes lead to an increase in frequency. However, this is far from universally true for any individual case. The best results have been obtained under strict regimes which are difficult to maintain. Perhaps its only therapeutic application lies in some degree of fluid restriction during febrile illnesses in epileptic children, and in instructions to all epileptics about the avoidance of excessive fluids of any sort.

Despite the suggestive improvement of some individuals under ketogenic diets, this is also not very impressive numerically, and again introduces a difficult and strict regime.

One word should be said about alcohol. Some individuals only have seizures during or following alcoholic bouts. All epileptics have inherently one formidable hazard—they are ill-advised from the standpoint of safety, or even the law, to add another hazard.

The foundation of the control of epileptic seizures lies in adequate drug therapy. This means the use of the most effective drug or combination of drugs in sufficient dosage, the avoidance of drug reactions, and finally the exhibition of the material at the time most needed. The choice of drugs has become an important matter and there are several from which to choose. No doubt the list of possibilities will increase. Though none are specific, certain drugs have been found empirically to be more useful than others in different manifestations of epilepsy. All have certain disadvantages or even dangers. All take a period of time in producing full clinical effect quite unrelated to rates of absorption and excretion. It is unwise, therefore, to judge the efficacy of a drug or dosage until the patient has been under its influence for a considerable period of time—usually a matter of weeks. Changes in type of drug or dosage must be made gradually. Secondly, the sudden withdrawal of one drug which is not achieving a completely satisfactory result, with or without the substitution of another, may initiate a striking recurrence of seizures—even precipitate a dangerous status epilepticus. These drugs must be tapered off as well as on. A third point of value lies in the timing of dosage. The administration of phenobarbital, for instance, to a patient with purely nocturnal attacks is more effectively made in the evening, with morning of course being a better choice for one with purely diurnal seizures. Similarly, increases in dosage may be made prior to menstrual periods if such have been known to be accompanied by increased seizure incidence.

Four drugs are now in common use in the treatment of epilepsy: the bromides, phenobarbital, dilantin, and tridione. Since they have been considered in detail by Dr. Butler, I propose merely to touch on certain clinical aspects of their use.

The bromides are now little used as a basic medication despite the tremendous advance made by their introduction some ninety years ago. Their effects are capricious and depend on associated chloride levels. They are cumulative and, with prolonged heavy dosage, can readily lead to evidence of toxicity—skin

lesions, mental dulling, emotional and psychotic changes. Nevertheless, the addition of bromides to other forms of treatment in cases which do not respond completely otherwise may be very effective. For such purposes in an adult, 0.6 gram doses, two, three, or four times a day are useful.

The introduction of phenobarbital added a much more powerful anticonvulsive drug. Its action is relatively rapid, and it can be given parenterally as well in the emergency treatment of status epilepticus. It is the most important routine antiepileptic drug, either by itself or in combination with dilantin. It is most effective in controlling the types of seizures associated with active motor or sensory manifestations—grand mal seizures, Jacksonian attacks, or focal epilepsy. Dosage of from 60 to 180 mg. daily (for an adult) is in most cases adequate, though sometimes it may be necessary to exceed this. Phenobarbital has limitations however. While it is well worth trying in petit mal attacks or akinetic seizures, the results are unpredictable. Some patients respond well, others do not. There is increasing evidence that its use in psychomotor episodes may even increase their frequency and this possibility must be borne in mind. Toxic effects are unusual with common therapeutic doses, though less rare with children. Larger doses may produce drowsiness, and this constitutes its greatest disadvantage. This can be counteracted, but only to some extent, with benzedrine or caffeine. Less common are mild ataxia or emotional changes. Rashes may rarely appear.

Dilantin, like phenobarbital, has its greatest effect on grand mal, Jacksonian, and focal seizures. Furthermore, evidence is accruing of a definite effect in the reduction of psychomotor attacks. It has the distinct advantage of being unassociated with mental clouding or drowsiness. It is generally used therefore as a basic medication in doses (for an adult) of 0.3–0.4 grams or more daily. It too, has certain limitations. The achievement of a dilantin effect is slower than that with phenobarbital. Some cases will respond more effectively to it than to phenobarbital—others, less well. Not infrequently its administration produces some degree of gastric distress so that its ingestion with meals is desirable. It is often indeed difficult to give it at other times. For these various reasons, its use is commonly augmented by smaller morning and/or evening doses of phenobarbital. Dilantin has, apart from the absence of drowsiness, more frequent toxic effects than phenobarbital. Skin eruptions sometimes occur. Enlargement of the gums may be distressing. Less commonly in adults, but not too infrequently in children, ataxia, diplopia, nystagmus, may appear.

The handling of patients suffering from seizures which are characterized essentially by lack or restriction of function, as opposed to those with striking motor and behavior changes, has always been difficult. I refer to petit mal and akinetic seizures. In both of these, the use of the above-mentioned drugs is often disappointing. Fortunately, the natural course of both is often a fluctuant one, and frequently one tending towards spontaneous improvement. This is especially true in children. However, this is far from the common experience. There is a real need for a drug with a specific effect on these forms. Tridione, which was

originally given clinical trial as an anticonvulsant material, has certainly met with some success in the treatment of both petit mal and akinetic attacks. The time has not yet come to assess its value adequately. In a recent report on 175 patients who had previously resisted all attempts at control, Lennox reported one-third as free of seizures, one-third as having less than one-quarter the previous number of attacks, one-third as unchanged. He used doses of 0.3 grams two, three, or four times daily. Other reports are somewhat less enthusiastic. Most clinicians who have used tridione have had the distressing experience, at one time or another, of finding major seizures appear for the first time in a patient with petit mal.

The toxic effects of tridione are likewise not yet clearly realized nor perhaps recognized. All patients under such therapy witness hemeralopia—an increased visual sensitivity to light—which may, or may not, be very distressing. Skin eruptions occur. Apparently the most dangerous effect is on the blood leucocytes. There is a tendency to neutropenia, and a check on this must be made frequently. At least three cases of fatal agranulocytosis have been reported so far. It seems, therefore, that in this drug we have a material which is more effective than other measures in the treatment of petit mal and akinetic seizures. It has, however, undesirable effects and dangerous possibilities. I think a wise plan at present is to reserve its use until the effects of all other measures, and of time, have been well assessed.

Dr. Harvey: Since Dr. Walker has been interested in the problem of focal epilepsy I will ask him to open the discussion.

Dr. A. Earl Walker: I have been particularly interested in focal epilepsy, i.e., convulsive seizures that develop due to a lesion in the cerebral cortex. The first question raised is “why do some people develop convulsive seizures when they have a brain lesion and others do not?” It has been suggested that a constitutional factor is responsible, a factor related to the cerebral dysrhythmia found in 15% of normal individuals. However, there is no clinical evidence for such a view. Ziskind studied patients with focal epilepsy and found no higher familial incidence of epilepsy among the relatives of such patients than in the normal population. Penfield and Erickson came to a similar conclusion. In the last war, in a series of 250 cases of post-traumatic epilepsy which we studied, the incidence of epilepsy in the families of the patients was no higher than in the general population.

We have been interested in the course of focal epilepsy. The attacks seemed to indicate that one part of the brain is responsible and that the attacks spread from that area. But the electro-encephalogram often indicates a generalized dysrhythmia. Is this the end result of a focal lesion or did the patient have a cerebral dysrhythmia originally? In practical terms, suppose a child of two or three years of age has a head injury and develops focal fits and at the age of ten years the electro-encephalogram looks like a generalized dysrhythmia, would removal of the original focus at the age of two or three have prevented the development of a generalized dysrhythmia?

To that question we have no definite answer, but there is some experimental evidence on this point. We have placed wax pellets of penicillin on the brain surface of monkeys, inducing a Jacksonian attack. After a short time the electro-encephalogram, instead of showing a focal abnormality, shows a generalized dysrhythmia; in other words electro-encephalographic alterations occur not only at the site of the pellet but also on the opposite side of the brain. The experimental epileptogenous focus may affect the entire brain so that it gives rise to a generalized dysrhythmia.

We encountered another problem in studying focal epilepsy. In many instances we did not see the attack and had only the patient's vague description of it. To determine the characteristics of the attack, focal or generalized, we attempted to activate the potential epileptogenous foci. Many techniques and drugs were tried but metrazol in small doses was found to reproduce best the clinical seizure. Moreover, 2 cc. of metrazol will cause electro-encephalographic attacks without inducing the clinical fit, so that the locus of the firing zone may be determined in relation to a cranial defect or scalp wound.

In the case of brain wounds, the focus lies about but never in the actual scar. One cannot elicit an attack by stimulation of the scar, but, if the appropriate part of the margin of the scar is stimulated, the attack occurs and is precisely the same as that which the individual has spontaneously. The focus determined by metrazol activation appears to be constant. It is in the same place today and tomorrow. Occasionally we found multiple epileptogenic foci.

Dr. Theodore Lidz: Can one safely assume if a dose of 2 cc. of metrazol is given that a normal individual will not have an attack, whereas an epileptic will?

Dr. Walker: The answer to that question is probably yes, although we do not have a large series of normal individuals. In posttraumatic epileptics, 2 cc. of a 10% solution of metrazol on a primary single test will give rise to convulsive manifestations in 45 per cent of cases. By repeating the test you can bring the percentage up to 67 per cent. There are 30 per cent of posttraumatic epileptics who will not have a seizure by this technique. In the idiopathic seizures the incidence is a little bit higher. Dr. Jasper has given the metrazol somewhat more slowly and can precipitate electro-encephalographic attacks in 85% of the cases of idiopathic epilepsy. Approximately 10% to 15% of the patients will have clinical generalized attacks, another 5% focal manifestations, the other 80% will have no clinical evidence of a fit. The chances are one in seven of producing a clinical seizure, whereas the chances of electro-encephalographic manifestations are eight in ten.

Dr. Leonard J. Gallant: There are some points I would like to bring up about the effect of drugs on the electro-encephalogram. First of all, with the average anticonvulsive doses of the medications mentioned there is no effect on the brain wave in normal people. In epileptics—I'd like to confine my remarks to the idiopathic kind—either they show no effects, or in most cases more abnormalities ap-

pear. Many larval attacks not shown spontaneously become more easily seen. With tridione, for example, we see an increase in the high voltage activity in the electro-encephalogram. Tridione will enhance the faster attacks which are like grand mal attacks. Any hypnotic given to the point of anesthesia enhances the abnormal components of the electro-encephalogram. Dilantin increases the slower component of the brain wave, and many of these people who are treated for grand mal, after treatment begin to show petit mal. Finally, since diagnosis is the first step in treatment I have felt epilepsy should be a clinical diagnosis, and the brain wave should be used as an adjunct because fifteen per cent of epileptics may not show any abnormality in the test and fifteen per cent of non-epileptics may show abnormalities.

Question: How often does hyperventilation precipitate grand mal in an epileptic subject?

Dr. Gallant: In one study the investigators kept the patients hyperventilating until a fit occurred. They found 37 per cent had seizures. Hyperventilation for three or four minutes will not generally precipitate convulsions in the epileptics. Hyperventilation for five to seven minutes will bring about convulsive episodes in a considerable number of patients.

Dr. Harvey: Would Dr. Woolsey care to make a few comments?

Dr. Clinton N. Woolsey: The subject as you have been considering it today has been clinical in its point of view. However, some recent results from the physiological laboratory may be pertinent and of ultimate practical interest for the surgical treatment and the diagnosis of epilepsy.

I suppose it is generally held that epileptic discharges from the cerebral cortex make use chiefly of the pyramidal tract and that this tract originates in the precentral motor cortex. There is increasing evidence that only a portion of the pyramidal tract originates in the so-called "motor cortex." We have recently applied a new technique to the study of this problem, and have undertaken to map the cortical origins of fibers traversing the medullary pyramid by stimulating the pyramid electrically and recording the potential changes produced in the cortex by the arrival of impulses conducted antidromically in the pyramidal axones. By this method it has been possible to confirm and extend the evidence regarding the regions of cortex contributing fibers to the pyramidal tract. The regions include areas four and six precentrally and the entire parietal lobe in the monkey and homologous areas in cat and rabbit.

The largest contribution apparently does indeed originate precentrally but there is also a very considerable part of the pathway which comes from the postcentral gyrus. Moreover, there is special interest in the pyramidal fibers whose cells are in that portion of the parietal cortex which forms the upper bank of the

sylvian fissure in the monkey and the homologous region in other species. This is the locus of a second somatic afferent area, so the region both receives and discharges. In the experimental animal discharges from this area can give rise to epileptiform convulsions of the Jacksonian type. Rasmussen and Penfield recently have reported data suggesting the existence of a similar system in man. Thus it should be important for diagnosis and surgical therapy to keep in mind the existence of efferent pathways not originating in the precentral motor cortex, especially since failure to consider this parietal motor system could lead to mistaken localization of an epileptogenic focus.

Dr. Harvey: There is still a great deal to be learned about epilepsy, nevertheless we are faced with the management of a large number of cases with a still inadequate armament. Emotional problems are important and I wonder if Dr. Lidz would care to comment.

Dr. Theodore Lidz: The psychiatric management of patients with epilepsy has been admirably discussed by Dr. Magladery. I should like to mention only one other point. When a patient has been rendered free of seizures by proper management and is apparently in a state of equilibrium, it sometimes happens that symptoms suddenly reappear. This recrudescence may be brought about by some minor emotional irritation on the part of the patient, and often superficial psychotherapy will effect a subsidence of symptoms. I would urge you to try such simple measures before undertaking extensive changes in drug regimens.

Dr. Gallant: Dr. Lidz' remarks lead to another interesting matter. Some epileptics may use their attacks for ego satisfaction. By preventing these attacks, the patient is robbed of this outlet and may develop other symptoms.

SUMMARY

The rational management of epilepsy depends on the separation of symptomatic seizures caused by brain tumors, syphilis, etc., from the "cryptogenic" variety. Only when specific causes can be excluded is it safe to proceed solely with measures to suppress the seizures. Such suppression is effected largely by drugs and by control of emotional factors. Secondary but important considerations are the avoidance of alcohol and of other situations wherein an attack would endanger the patient or others. Dehydration and ketogenic diets have infrequent usefulness in the modern therapy of epilepsy.

Emotional and social rehabilitation is one of the key points in the proper management of epileptics. Such factors play an important role in the precipitation and in the frequency of attacks. More serious is the impact of repeated attacks on the psychological development and adjustment of the individual to school, work, and social situations.

The drugs of greatest usefulness are phenobarbital and dilantin. Bromides are not much used because of toxicity. Tridione has been successful in the suppression of petit mal and akinetic seizures, but it occasionally causes agranulocytosis and has other unpleasant side effects. Methods of animal screening have been improved and it is to be hoped that more potent and less toxic agents will be forthcoming. Animal experimentation has demonstrated also that a focal lesion of the cortex may give rise later to a generalized dysrhythmia, and secondly that cortical areas other than those of the motor cortex participate in motor phenomena.

PHYSIOLOGICAL STUDIES IN CONGENITAL HEART DISEASE¹

V. THE CIRCULATION IN PATIENTS WITH ISOLATED SEPTAL DEFECTS

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In three of the preceding papers of this series various types of congenital heart disease have been discussed (1, 2, 3). This report presents the physiological studies made in a group of individuals with isolated septal defects. Five cases with these lesions were selected as being most representative of the various clinical aspects of this anomaly. These cases will be presented in a sequence of increasing clinical disability to clarify the relationship between the degree of incapacity and the physiological data obtained.

METHODS

The methods used in these studies have been described in detail and evaluated in the first paper of this series (4). The Fick principle has been applied to determine the blood flow through various parts of the circulation. A restatement of the formulae based upon this principle and a definition of terms is made at this point for clarity.

a. Systemic flow is the volume of blood passing through the peripheral vessels per minute.

$$\frac{\text{Systemic Blood Flow (ml. per minute)} = \text{O}_2 \text{ uptake (ml. per minute)} \times 100}{\text{O}_2 \text{ content of peripheral arterial blood (volume per cent)} - \text{O}_2 \text{ content right auricular blood (volume per cent)}}$$

b. Pulmonary artery flow is the volume of blood passing through the pulmonic valve into the pulmonary artery per minute.

$$\frac{\text{Pulmonary Artery Flow (ml. per minute)} = \text{O}_2 \text{ uptake (ml. per minute)} \times 100}{\text{O}_2 \text{ content of pulmonary vein blood (volume per cent)} - \text{O}_2 \text{ content pulmonary arterial blood (volume per cent)}}$$

¹ Supported by grants from the Commonwealth Fund and the Carolyn Rose Strauss Foundation.

If the oxygen content of pulmonary vein blood is not measured directly, it is assumed to be 95 per cent of the oxygen capacity. If blood of the pulmonary artery is not obtained, a sample taken from the right ventricle near the pulmonic valve (the outflow tract) is substituted.

c. From a and b it may be computed that

Shunt (ml. per minute) right to left = Systemic flow — Pulmonary artery flow
or

Shunt (ml. per minute) = left to right = Pulmonary artery flow — Systemic flow

The formulae permit an estimation of the volume and overall direction of the intracardiac shunt. Nevertheless, an analysis of the oxygen contents gives evidence that reciprocal admixture does occur.

d. Effective pulmonary flow is that volume of mixed venous blood which, after its return to the right auricle, ultimately reaches the pulmonary capillaries.

$$\frac{\text{Effective pulmonary flow (ml. per minute)} = \text{O}_2 \text{ uptake (ml. per minute)} \times 100}{\text{O}_2 \text{ content of pulmonary vein blood (volume per cent)} - \text{O}_2 \text{ content right auricular blood (volume per cent)}}$$

Bloods for analysis from the various parts of the circulatory system were obtained by cardiac catheterization, following the method of Cournand (5), and by direct arterial puncture. Blood gas analyses were carried out in the manometric apparatus of Van Slyke and Neill (6). Arterial and intracardiac pressures were optically recorded by means of a Hamilton manometer (7), and mean pressures were calculated by planimetric integration of the area under the curve. Respiratory gases were collected in Douglas bags and analyzed in the Haldane apparatus (8) and corrected to standard temperature and pressure. Resistance in the pulmonary circuit was calculated by Aperia's formula (9). The standard exercise test referred to is described in an earlier communication (4), and its interpretation and significance therein discussed.

The shortcomings inherent in the application of the above formulae have been discussed in connection with the determination of pulmonary artery flow in the presence of a ventricular defect (4). Similarly, the calculation of systemic flow is complicated by reciprocal admixture through an auricular septal defect. This necessitates the use of the oxygen content of blood from the superior vena cava as representative

of mixed venous blood. This may provide an error in the results, since it has been shown that true mixture of venous blood does not occur before the outflow tract of the right ventricle (5).

All the cases presented in this report fulfill the physiological criteria for the presence of septal defects, although in none has the diagnosis been confirmed by postmortem examination (10, 11). An auricular septal defect is present when the oxygen content of the right auricular blood exceeds that of blood from superior vena cava blood by about 2 volume per cent. A similar difference between right auricular and right ventricular blood demonstrates the presence of a ventricular septal defect. Direct evidence of such defects may be obtained when the left chambers of the heart are catheterized.

Case 1. A. G. A. (age 10, date of study November 12, 1947). The birth and development of this child were normal. When the patient was 2 months old the family physician diagnosed heart disease. At the age of two years he had an episode of slight cyanosis and momentary loss of consciousness. Two similar episodes followed within six months, but there were no further recurrences. At the time of examination he suffered no incapacity.

On physical examination there was no clubbing or cyanosis. The positive findings were limited to the heart. The blood pressure in the arm was 105/70. There was a marked thrill over the precordium, maximal in the left second interspace. There was a very loud, widely transmitted systolic murmur over the precordium, maximal in the second and third interspace, and a long, blowing, much fainter diastolic murmur along the left sternal border.

On fluoroscopy, the heart was of normal size, with a prominent pulmonary conus. Pulmonary pulsations of normal degree were seen. By x-ray, some right auricular enlargement was seen in the LAO position. The ECG was normal. Routine laboratory studies revealed a hematocrit of 38, hemoglobin of 12.0 grams, and a red count of 4.6 million.

Physiological studies: The standard exercise test was not performed. Pertinent data obtained during cardiac catheterization are shown in Table I. Significant findings are as follows:

1. The oxygen content of right ventricular blood exceeded that of right auricular blood by 4.6 volume per cent. The oxygen content of pulmonary arterial blood was 3.4 volume per cent higher than that of right auricular blood.
2. The oxygen saturation of peripheral arterial blood was 99 per cent.
3. The pulmonary arterial pressure was 30/10, with a mean pressure of 20 mm. Hg.

Comment: The rise in oxygen content from right auricular to right ventricular blood indicates the presence of a ventricular septal defect allowing admixture of oxygenated blood with the venous blood. The

slightly lower oxygen content of the pulmonary arterial blood may be due to the fact that the right ventricular sample was taken as the catheter tip lay near the septal defect. The overall shunt is 5260 cc./min./M₂ directed from left to right as shown by the fact that the pulmonary artery flow exceeds the systemic flow.

The full saturation of the peripheral arterial blood, as well as the equality of systemic and effective pulmonary flow, indicate that the shunt is directed entirely from left to right with no reciprocal admixture (Fig. 1).

TABLE 1
Date obtained from right heart catheterization

CASE NUMBER	DATE	NAME	SEX	AGE	SURFACE AREA (M ₂)	O ₂ CONSUMPTION (cc.)	CO ₂ PRODUCTION (cc.)	RQ	MINUTE VOLUME (L/M)	BAR	SVC O ₂ (VOL. %)	RA O ₂ (VOL. %)	RV O ₂ (VOL. %)	PA O ₂ (VOL. %)	PV O ₂ (VOL. %)	ARTE- RIAL BLOOD		CARDIAC OUTPUT		SHUNT (L/M/M ₂)	EFFECTIVE (L/M/M ₂)
																Capacity (vol. %)	Saturation (%)	Pulmonary (L/M/M ₂)	Systemic (L/M/M ₂)		
1	11/12	AGA	M	10	0.93	128	103	0.79	3.7	-18	13.8	12.6	17.2	16.0	17.8	17.9	99.0	7.96	2.70	5.26 L-R	2.7
2	9/30	AW	M	9	1.16	154	67	0.37	5.2	-23	13.6	13.9	16.3	15.4	18.8	19.8	91.0	3.91	3.24	0.67 L-R	2.7
3	10/28	VE	F	27	1.18	122	100	0.81	5.0	-18	12.0	15.7 14.5	17.7	15.0 15.7	19.5	20.5	90.0	2.50	1.65	0.86 L-R	1.4
4	9/16	GD	M	16	1.72	252	248	1.0	11.4	+8	15.9	17.9		18.7	24.3	25.6	79.5	2.62	3.33	0.71 R-L	1.7
5	4/2	LG	M	30	2.0	145	167	1.16	6.8	-21	19.1	20.8	20.8	20.7	32.0	33.6	70.5	1.23	4.78	3.55 R-L	1.2

Case 2. A. J. W. (age 9, date of study September 30, 1947). This child was apparently normal at birth. Except for a single attack of unconsciousness with convulsions at the age of three months, his development was unremarkable. When he was five days old the family physician noted that he had a cardiac murmur. For six months prior to admission he had occasional precordial pain, unrelated to exercise. Slight dyspnea began at this time and subsequently increased, although it remained moderate and caused the patient no apparent incapacity.

On physical examination there was no clubbing or cyanosis. The positive findings were limited to the heart. The blood pressure in the arm was 102/48. The cardiac outline was normal to percussion. There was a systolic thrill distributed over the entire anterior chest wall. In the left first, second and third interspaces a continuous murmur could be heard, but the diastolic component

was much less pronounced than the systolic. On fluoroscopy the heart was of normal size with a prominent pulmonary conus. The vascular markings in the lung fields were increased, but pulsations were normal. On x-ray, both the right auricle and ventricle were seen to be enlarged. The ECG showed left axis deviation and normal left ventricular preponderance. Routine laboratory studies revealed a hematocrit of 38.5, a hemoglobin of 11.9 grams, and a red count of 4.5 million.

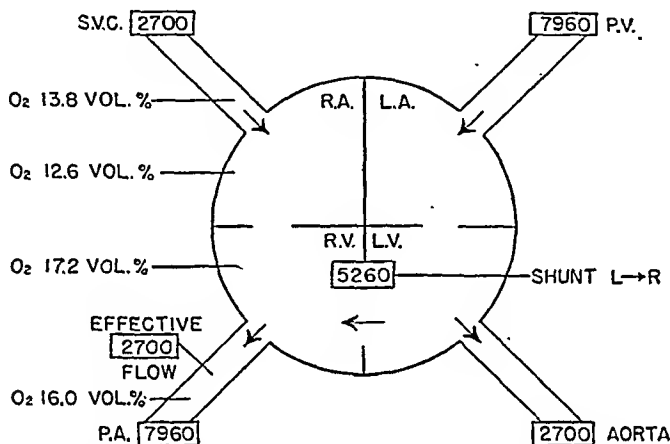


FIG. 1. ILLUSTRATING THE FINDINGS IN CASE 1 (A. G. A.)

The oxygen content of right ventricular blood is significantly higher than that of the right auricular blood, indicating a ventricular septal defect. The pulmonary artery flow exceeds the systemic flow, giving the intracardiac shunt an overall direction of left to right. Since the effective pulmonary flow equals the systemic flow, there is no reciprocal right to left admixture.

Physiological studies: The standard exercise test was not performed. Pertinent data obtained during cardiac catheterization are shown in Table I. Significant findings are as follows:

1. The oxygen content of the right ventricular blood was 2.4 volume per cent higher than that of the right auricular blood.
2. The saturation of the peripheral arterial blood was 91 per cent.
3. The pulmonary artery flow was greater than the systemic flow.
4. The pulmonary arterial pressure was 40/29 with a mean pressure of 34 mm. Hg.

Comment: The rise in oxygen content from right auricular to right ventricular blood indicates the presence of a ventricular septal defect, allowing admixture of oxygenated with venous blood. The overall shunt is 670 cc./min./M₂ directed from left to right, as shown by the

fact that the pulmonary artery flow exceeds the systemic flow. However, reciprocal right to left admixture is demonstrated by the lowered saturation of peripheral arterial blood and by the fact that the effective pulmonary flow was less than the pulmonary artery flow (Fig. 2).

Case 3. V. E. (age 27, date of study October 20, 1947). This patient was a Lorain type dwarf. Her birth was not remarkable. Her developmental history was normal except for lack of growth. For as long as she could remember she had known she had heart disease. Although she had had some exertional dyspnea

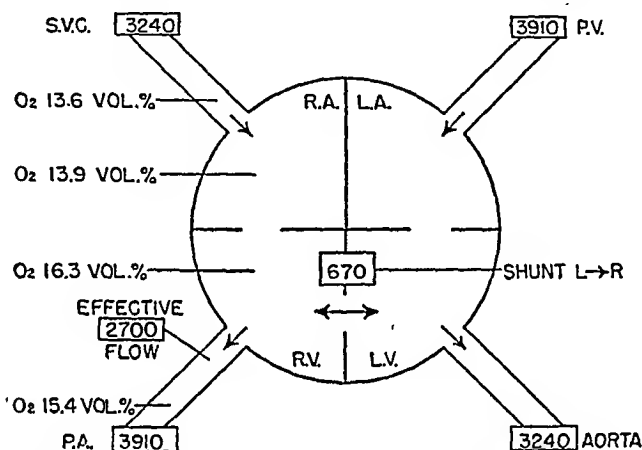


FIG. 2. SHOWING THE FINDINGS IN CASE 2 (A. J. W.)

The oxygen content of right ventricular blood is significantly higher than that of the right auricular blood, indicating a ventricular septal defect. The pulmonary artery flow exceeds the systemic flow, giving the intracardiac shunt an overall direction of left to right. Since the effective flow is lower than the systemic flow, there is reciprocal admixture from right to left.

while in school, it had not been progressive, nor had she ever manifested cyanosis. In 1933 she became pregnant and, suffering some increase in her dyspnea, was hospitalized. The child was delivered by Caesarean section. An ECG in March of that year showed left axis deviation and myocardial damage. The patient was not seen again until January 1947, at which time she was having increasing exertional and nocturnal dyspnea. Her liver and spleen were enlarged. She was digitalized, but continued to suffer intermittent bouts of decompensation.

On physical examination at the time of the present study, the positive findings were limited to the heart, except for the traits of dwarfism. There was no clubbing or cyanosis. The blood pressure in the arm was 110/70: The heart was enlarged to the left. There was a loud systolic murmur and thrill in the left second inter-space, transmitted all over the chest. At the apex there was a harsh, high pitched

systolic blow. There were no diastolic murmurs. X-ray, on September 11, 1947, revealed the heart to be enlarged to the left. The pulmonary artery was prominent and the aorta hypoplastic. Slight enlargement of the right auricle and right ventricle was noted. The ECG showed left axis deviation and bundle branch block. Laboratory studies revealed a hematocrit of 45.5, a hemoglobin of 13.8 grams, and a red count of 4.9 million. Circulation time (decholin) was 10 seconds.

Physiological studies: The standard exercise test showed a rise in oxygen consumed per liters of ventilation (Table II). Pertinent data obtained during cardiac catheterization are shown in Table I. Significant findings are as follows:

1. The oxygen content of the right auricular blood was 2.5 volume per cent higher than that of the blood of the superior vena cava. The oxygen content of the right ventricular blood was 2.0 volume per cent higher than that of the right auricular blood.

TABLE II
Results Obtained from the Standard Exercise Test

CASE NO.	DATE	NAME	SEX	AGE	OXYGEN CONSUMED PER LITER VENTILATION (CC.)		CO ₂ PRODUCED PER LITER VENTILATION (CC.)
3	9/11/47	VE	F	27	Rest	21.5	17.8
					Exercise	24.3	19.7
4	7/17/47	GD	M	23	Rest	16.1	19.6
					Exercise	17.5	18.0
5	3/11/47	LG	M	30	Rest	21.3	24.5
					Exercise	46.0	23.7

2. The oxygen saturation of the peripheral arterial blood was 90 per cent.

3. The pulmonary arterial blood flow exceeded the systemic flow.

4. The pulmonary arterial pressure was 49/20 with a mean pressure of 39 mm. Hg.

Comment: The rise in oxygen content of blood from superior vena cava to right auricle indicates the presence of an auricular septal defect allowing left to right admixture of blood. A further rise in oxygen content of the blood from right auricle to right ventricle indicates the presence of a ventricular septal defect. The overall shunt is 855 cc./min./M₂ directed from left to right as shown by the fact that the pulmonary artery flow exceeds the systemic flow and that the effective pulmonary flow is lower than pulmonary artery flow. Re-

ciprocal right to left admixture through the defects is indicated by the lowered saturation of peripheral arterial blood.

In the calculation of the systemic flow, the oxygen content of blood from the superior vena cava was used. This resulted in an overall shunt directed to the right. If higher oxygen content of blood were substituted in the Fick equation, the calculated systemic flow would exceed the pulmonary artery flow. From this calculation the overall

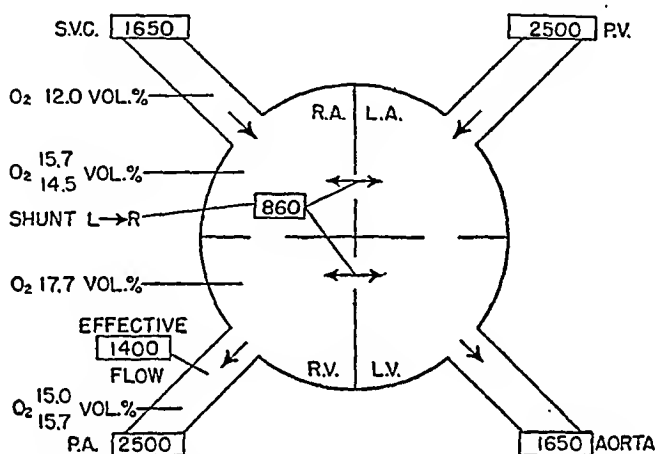


FIG. 3. SHOWING THE FINDINGS IN CASE 3 (V. E.)

The oxygen content of the right auricular blood is significantly higher than that of the superior vena cava. The oxygen content of the right ventricular blood is significantly higher than that of the blood of the right auricle. Auricular and ventricular septal defects are thereby indicated. The pulmonary artery flow exceeds the systemic flow, giving the intracardiac shunt an overall direction of left to right. Since the effective flow is lower than the systemic flow, there is reciprocal admixture from right to left.

shunt would be directed toward the left. It is evident, therefore, that calculation of shunts in patients with septal defects, except for their order of magnitude, are at best approximations (Fig. 3).

Case 4. G. D. (age 16, date of study September 16, 1947). The patient's birth and development were normal. When he was two years old his parents were informed by a physician that he had a "cardiac condition." When he was ten his parents first noted marked dyspnea and mild cyanosis on moderate exertion. Despite a progression of these symptoms, he had no convulsions, paralysis or loss of consciousness. Although he could walk half a mile, climbing stairs caused extreme dyspnea.

On physical examination the patient was seen to be mildly cyanotic with slight clubbing of the fingers. The blood pressure in the arm was 104/78. The thorax was asymmetrical, bulging slightly on the left side. The heart was within normal limits of size to percussion. There was a rough, moderately loud systolic murmur heard best at the left sternal border in the fourth interspace and transmitted to the apex. The second pulmonic sound was snapping and was louder than the second aortic sound. On fluoroscopy the size of the heart was normal. The pulmonary conus was prominent and the pulmonary artery was large and quiet. The ECG showed right axis deviation and right ventricular hypertrophy with a slight delay in interventricular conduction time. Laboratory studies revealed a hematocrit of 43.5, a hemoglobin of 14 grams, and a red count of 5.3 million.

Physiological studies: The standard exercise test showed a rise in the oxygen consumed per liters of ventilation (Table II). Pertinent data obtained during cardiac catheterization are shown in Table I. Significant findings are as follows:

1. The oxygen content of the right auricular blood was 2.0 volume per cent higher than that of the blood of the superior vena cava.
2. The oxygen saturation of the peripheral arterial blood was 79.5 per cent.
3. The systemic blood flow exceeded the pulmonary artery flow.
4. The pulmonary arterial pressure was 74/47 with a mean pressure of 64 mm. Hg.

Comment: The rise in oxygen content of blood from the superior vena cava to right auricle indicates the presence of an auricular septal defect. The overall shunt is 710 cc./min./ M_2 directed from right to left as shown by the fact that the systemic flow exceeds the pulmonary artery flow. The low saturation of the peripheral arterial blood and the fact that the effective pulmonary flow is lower than the pulmonary artery flow give further evidence of the right to left shunt. However, the rise in oxygen content from superior vena cava to right auricular blood shows reciprocal admixture from left to right through the auricular septal defect (Fig. 4).

Case 5. L. G. (age 30, date of study April 2, 1947). The patient recalls only that his mother restricted his activity and that he had two episodes of epistaxis during his childhood. He was supposed to have been cyanotic at birth and during childhood, and to have suffered easy fatigue and dyspnea. As far back as he can recall, he has had some degree of cyanosis of the nailbeds and lips aggravated by exertion and cold. He became dyspneic on mild exercise.

On physical examination, slight clubbing and cyanosis were noted. The remaining positive findings were limited to the heart. The blood pressure in the left arm was 90/76; in the right arm, 112/92. The heart was normal in size to percussion. There was no thrill. There was a short blowing systolic murmur

at the lower left sternal border. In the fifth interspace at the sternal edge a short high-pitched systolic murmur was well localized. Neither murmur was transmitted. On fluoroscopy the size of the heart was normal. There were increased vascular markings with minimal pulsations in the lung fields. The pulmonary arteries were large. The ECG showed right axis deviation, right ventricular hypertrophy, and right bundle branch block. Laboratory studies revealed a hematocrit of 71.2, a hemoglobin of 21.8 grams, and a red count of 9.8 million. Angiocardiography gave equivocal results.

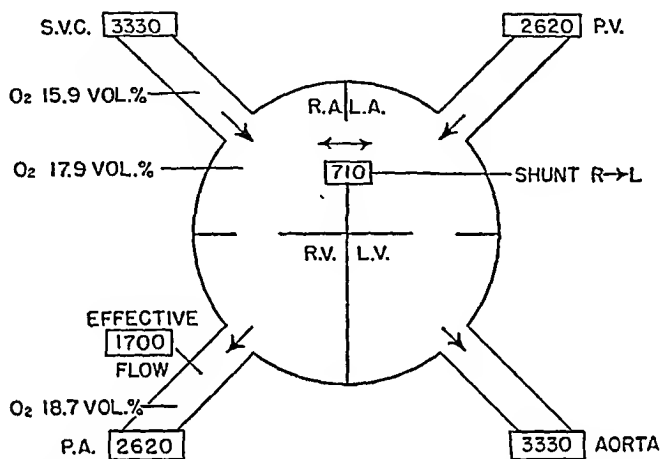


FIG. 4. ILLUSTRATING THE FINDINGS IN CASE 4 (G. D.)

The oxygen content of the right auricular blood is significantly higher than that of the superior vena cava blood. This indicates the presence of an auricular septal defect. The systemic flow exceeds the pulmonary artery flow, giving the intracardiac shunt an overall direction of right to left. Since the effective flow is lower than the pulmonary artery flow, there is reciprocal admixture from left to right.

Physiological studies: The standard exercise test showed a rise in the oxygen consumed per liters of ventilation (Table II). Pertinent data obtained during cardiac catheterization are shown in Table I. Significant findings are as follows:

1. The oxygen content of the right auricular blood was 1.7 volume per cent higher than the blood from the superior vena cava.
2. The oxygen saturation of the peripheral arterial blood was 70.5 per cent.
3. The systemic blood flow exceeded the pulmonary artery flow.
4. The pulmonary arterial pressure was 103/70 with a mean pressure of 82 mm. Hg.

Comment: The rise in oxygen content of blood from superior vena cava to right auricle indicates the presence of an auricular septal defect

through which left to right admixture is occurring. However, the overall shunt of 3550 cc./min./M₂ is directed from right to left as shown by the fact that the systemic flow exceeds the pulmonary artery flow. Further manifestations of this right to left shunting are found in the reduced peripheral arterial saturation, and in the fact that the effective pulmonary flow is lower than the pulmonary artery flow (Fig. 5).

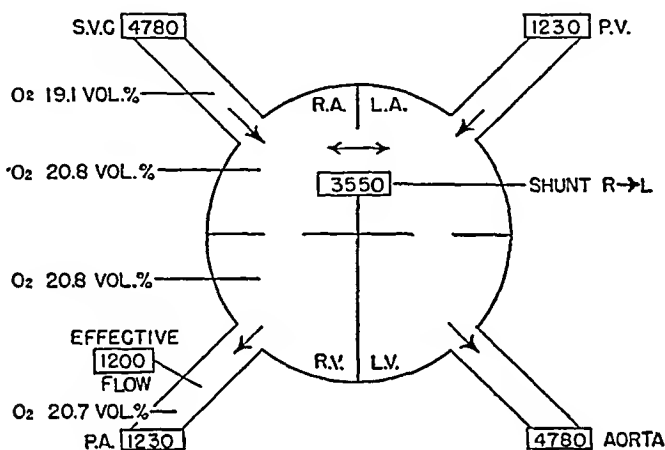


FIG. 5. SHOWING THE FINDINGS IN CASE 5 (L. G.)

The oxygen content of the right auricular blood is significantly higher than that of the superior vena cava blood. This indicates the presence of an auricular septal defect. The systemic flow exceeds the pulmonary artery flow, giving the intracardiac shunt an overall direction of right to left. Since the effective flow is lower than the pulmonary artery flow, there is reciprocal admixture from left to right.

DISCUSSION

A review of the histories of the five patients presented in this report reveals that all had cardiac disease since childhood. Their commonest presenting complaints were exertional dyspnea and intolerance to exercise.

Physical examination showed certain pertinent facts. Two of the patients exhibited cyanosis and digital clubbing. In only one instance (Case 3, V. E.) was the heart enlarged to percussion. All five patients had systolic murmurs of varying degrees, intensities, distribution, and transmission. Two patients (Case 1, A. G. A.; Case 2, A. J. W.)

had diastolic murmurs at the second and third interspace at the left sternal border. On fluoroscopy, only one patient (Case 3, V. E.) had cardiac enlargement, but a prominent pulmonary conus was revealed in three patients (Case 1, A. G. A.; Case 2, A. J. W.; Case 4, G. D.). The ECG was normal in the first patient, showed right axis deviation in the patients with auricular septal defects (Case 4, G. D.; Case 5, L. G.) and left axis deviation in those with ventricular septal defects (Case 2, A. J. W.; Case 3, V. E.). Laboratory data revealed normal hematocrits, hemoglobins, and red counts in all but one patient (Case 5, L. G.) who had a marked polycythemia.

As part of the physiological studies conducted in this group, three of the patients performed the standard exercise test. In all there was a rise in the oxygen consumed per liter of ventilation (Table II). This finding is in contrast with the results obtained in the study of patients with tetralogy of Fallot (1). In these latter there was generally a fall in the ratio of oxygen consumed per liter of ventilation during exercise. Conversely, in the present group under study, as in normal individuals and in patients with the Eisenmenger complex (2) the rise in oxygen consumed per liter of ventilation during exercise demonstrates that the effective pulmonic blood flow can increase significantly with exercise.

The other physiological studies showed changes paralleling the variations in clinical findings. A comparison of the blood flows revealed that three patients (Case 1, A. G. A.; Case 2, A. J. W.; Case 3, V. E.) had shunts which were predominantly from left to right. Two patients showed overall right to left shunts (Case 4, G. D.; Case 5, L. G.). In these two latter patients cyanosis was marked and the saturation of the peripheral arterial blood was low (Table I).

Pulmonary factors concerned with oxygen transfer in the lung are apparently not involved in this decrease in the saturation of peripheral arterial blood. In 16 cases in which it was possible to catheterize the pulmonary vein, the blood returning to the heart from the lungs was fully saturated. Therefore, arterial unsaturation is probably due to the reduction in the percentage of mixed venous blood perfusing the lung, which results from intracardiac shunting from right to left (12).

The intracardiac shunting of blood is expected to be predominantly directed to the right in the presence of a normal total cross sectional

area of the pulmonary arterial bed and in the absence of pulmonic stenosis. The finding in two of these cases that the shunt is predominantly directed to the left, implies an increase in the resistance of the pulmonary vascular tree. In this respect these cases show a disturbance of their pulmonary hemodynamics comparable to that seen in Eisenmenger's complex (2).

Two other observations support the finding of increased pulmonary resistance. First, the blood pressure in the pulmonary artery is increased in both systolic and diastolic components (Table III). Second, calculation of the loss of pressure head in the pulmonary

TABLE III
Intracardiac and Peripheral Blood Pressures. Resistances in Pulmonary and Peripheral Circulation

CASE NO.	DATE	NAME	SEX	AGE	FEMORAL ARTERY			SYSTEMIC FLOW (L/M/SEC.)	RESISTANCE (DYNES/CM ⁵ /SEC.)	RV		PA			PA FLOW (L/M/SEC.)	RESISTANCE (DYNES/CM ⁵ /SEC.)
					Systolic	Diastolic	Mean			Systolic	Diastolic	Systolic	Diastolic	Mean		
1	11/12	AGA	M	10	105	61	76	2.7	2,252	32	8	30	10	20	7.9	197
2	9/30	AW	M	9	155	71	106	3.2	2,650	40	29	40	29	34	3.9	695
3	10/28	VE	F	27	142	87	107	1.7	5,050	72	3	49	20	39	2.5	1,245
4	9/16	GD	M	23	136	88	103	3.3	2,470	74	32	74	47	64	2.6	1,970
5	4/2	LG	M	30	100	73	77	4.8	1,290	103	0	103	70	82	1.2	5,450

circuit reveals that the greatest elevation of resistance occurs in patients with right to left shunts (Table III).² In one of these cases (5, L. G.) the resistance approaches the magnitude of that seen in Eisenmenger's complex.

The increase in resistance is further reflected in the work of the right ventricle. The subject of the work of the heart and the assumptions necessary for its computation have been discussed in a previous paper of this series (2). Table IV demonstrates that the relative work of the two ventricles changes as the pulmonary resistance increases. The significant fact which appears from these

² Normal resistance = 480 dynes/cm.⁵/sec. Calculated according to Aperia's formula (9) using a normal mean pulmonary arterial pressure of 18 mm. Hg (13) and normal cardiac index.

calculations concerns the ratio of the velocity energy of the ventricle to its total work.³ In normal individuals the ratio, velocity energy/total work, for the right ventricle is five times as great as this ratio for the left. Table IV shows that only in Case 1 (A. G. A.) is the ratio within normal range. In Case 2 (A. J. W.) and Case 4 (G. D.) the ratio is 3/1 and 1/1 respectively. It would seem, therefore, that an increasing amount of the energy of the right ventricle is dissipated in overcoming the increased resistance in the pulmonary vascular tree.

Thus, evidence of increased resistance in the pulmonary vascular circuit is furnished by four findings: (1) intracardiac shunting of blood from right to left; (2) the presence of pulmonary arterial hypertension; (3) the marked loss of pressure head in the pulmonary circulation which becomes apparent when resistance is calculated; and (4) the changes in the ratio, velocity energy/total work, of the two ventricles.

The causes of increased pulmonary resistance in cases of septal defect have been discussed by a series of investigators. First, the underlying mechanism for predominant left to right shunting has been elucidated. Uhley proposed in 1942 that gravity alone was the cause for the direction of this shunt. This investigator doubted explanations which were based upon differences in auricular pressures (15). However, recent work by Brannon, Weens, and Warren (10), and by Cournand and his group (16) has demonstrated by direct measurements that the left auricular pressure exceeded the right in cases in which both auricles were catheterized. These studies indicate that flow through the septal defect is ordinarily from left to right because of this pressure gradient.

* The formulae used in these calculations are after Starling (14):

(1) Pressure Energy (gram-cm.) = $MP \times SD \times D \text{ Hg.}$

a. MP = Mean pressure in pulmonary artery or aorta.

b. SD = Systolic discharge (cc./minute) = $\frac{\text{Min. vol. (cc. min.)}}{\text{Pulse rate.}}$

c. D Hg. = Specific gravity mercury/10.

(2) Velocity Energy (Gram-cm.) = $Va \times SD/2g.$

a. g = gravity constant (980)

b. Va = Velocity of blood in pulmonary artery or aorta (cm./sec.)

i. $Va = SD / Ca \times ET$

A. Ca = Cross section (cm.²) pulmonary artery or aorta

B. $ET = \text{Systolic ejection period (sec)} = \frac{60/\text{pulse rate}}{8/3}$

TABLE IV
The Work of the Heart in Three Cases of Septal Defect

CASE NUMBER	DATE	NAME	SEX	AGE	RIGHT HEART										LEFT HEART										Ratio Rt. Vel./Lt. Work
					Systolic Discharge (cc.)	Cross Section Area (cm. ²)	Systolic Ejection Time (sec.)	Velocity (cm./sec.)	Velocity Energy (gram-cm.)	Pressure Energy (gram-cm.)	Mean Pressure (mm. Hg)	Total Work (gram-cm.)	Velocity Energy Total Work × 100	Systolic Discharge (cc.)	Cross Section Area (cm. ²)	Systolic Ejection Time (sec.)	Velocity (cm./sec.)	Velocity Energy (gram-cm.)	Pressure Energy (gram-cm.)	Mean Pressure (mm. Hg)	Total Work (gram-cm.)	Velocity Energy Total Work × 100			
*Norm.					66.5	5.14	0.3	43.0	1.46	1360	15	1361.5	0.1	66.5	3.9	0.3	57	1.94	9050	100	9051.9	0.02	5		
1	11/12	GA	M	10	82.4	5.14	0.25	64.0	2.7	2241.3	20	2244	0.1	28	3.9	0.25	27	0.38	2894.1	76	2894.5	0.013	10		
2	9/30	AW	M	9	52.2	5.14	0.27	37.6	1.0	2413.7	34	2414.7	0.041	44.2	3.9	0.27	42.1	0.94	6371.9	106	6372.8	0.015	3		
4	9/16	GD	M	23	52.9	5.14	0.27	38.3	1.0	4604.4	64	4605.4	0.021	67.4	3.9	0.27	64.2	2.2	9449.4	103	9451.6	0.023	1		

* Values for the work of the normal heart were derived using minute vol. = 5000 cc./min.; pulse rate = 75/min.; cross sec. PA = 5.14 cm.²; cross section aorta = 3.9 cm.²; mean PA pressure = 15 mm. Hg; mean pressure aorta = 100 mm. Hg.

A second approach to the problem of increased pulmonary resistance has dealt with the effects of increased blood flow. Levy and Blalock (17) in experimental observations on dogs with artificial patent ducti arteriosi, and Vandam, Bing, and Gray (3) studying patients with patent ducti arteriosi reach similar conclusions. Their findings suggest that the pulmonary vascular bed can normally accommodate a large rise in volume flow with no rise in the pressure of the peripheral pulmonary vessels. It is apparent, therefore, that normally pulmonary resistance is low.

In the light of these observations, the assumption may be ventured that the increase in pulmonary resistance results from changes in the pulmonary vascular tree. Posselt, in 1909, examined the pulmonary vessels in cases of septal defects and noted sclerotic changes in the large arteries (18). Brenner also noted sclerotic changes in the larger pulmonary arteries in routine autopsies. He did not believe that these were sufficient to increase pulmonary resistance (19). More recently, changes affecting the smaller radicles of the pulmonary vascular tree have been described. Taussig, Harvey, and Follis (20), Burrett and White (21), Johannsen and Connor (22), and Bedford, Parkinson, and Papp (23) have reported these changes in cases of septal defects which have been examined postmortem. However, no attempt has been made to correlate this finding with pulmonary hypertension. One might conjecture that widespread sclerotic changes affecting the smaller vessels, possibly in combination with thrombi, could raise pulmonary resistance. It is impossible to state at this time whether or not such changes are a result of increased pulmonary artery flow or develop as a result of other factors.

On the other hand, the rise in pulmonary resistance may result from increased left auricular pressure rather than from changes in the pulmonary vascular tree. The proof of this must await a method for simultaneous measurement of the pressure in both auricles and in the pulmonary artery. It is doubtful that this mechanism is at work in the cases of this series, since pulmonary congestion which would be expected to accompany such a condition is absent.

In addition to these mechanisms, functional factors have been considered in elevating pulmonary resistance (24, 25). Further investigation is necessary to elucidate this problem.

SUMMARY

Five cases of septal defects have been presented.

In the three cases in which the standard exercise test was performed the consumption of oxygen per liter of ventilation rose.

In three patients the overall direction of the intracardiac shunt was from left to right, and in two the shunt was from right to left. The degree of clinical disability paralleled an increase in pulmonary resistance. Increased resistance in the pulmonary bed was evidence by (a) right to left shunt; (b) elevation of systolic and diastolic pressures in the pulmonary artery; (c) marked loss of pressure head in the pulmonary vascular tree; and (d) changes in the ratio, velocity energy/total work, of the two ventricles. Possible mechanisms for the rise in pulmonary resistance were discussed.

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PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD HALL, MARCH 8, 1948

*The Experimental Pharmacology and Effectiveness of 3-4, Dimethyl-5-sulfanilamido-isoxazole.** DRs. MORTON S. BRYER, EMANUEL B. SCHOENBACH, C. EARL OTT and PERRIN H. LONG (*Department of Preventive Medicine, The Johns Hopkins University School of Medicine*).

3-4, Dimethyl-5-sulfanilamido-isoxazole is a sulfonamide derivative first described by Schnitzer et al (1). Reports of its use in urinary tract infections have appeared (2, 3). This short report deals with the experimental pharmacology and effectiveness of this sulfonamide derivative.

The L. D. 50 of this sulfonamide derivative for Swiss white mice is approximately 3.5 grams per kilogram when given as the sodium salt by the subcutaneous route. When a single dose of 1.25 grams per kilogram suspended in accacia is administered per os to mice, the average total concentration of the drug in the blood of these animals is 8 mgms. % at 1 hour, 3 mgms. % at 2 hours and 2 mgms. % at 4 hours. In man, following an initial dose of 3.0 grams and then 1.0 gram of the drug q. 4 hours for 48 hours, average concentrations of the drug in the blood were as follows: in 4 hours 4.8 mgms. %; in 8 hours 4.2 mgms. %; in 24 hours 3.7 mgms. %; in 32 hours 3.6 mgms. %; and in 48 hours 4.1 mgms. % respectively. In three test subjects, each of whom received an initial dose of 3.0 grams of this compound and then maintenance doses of 1.0 gram every 4 hours for 48 hours, 46, 51, and 55 per cent of the drug was excreted in a period of 96 hours after the first dose. In man little of the drug was found to be acetylated either in the blood or urine during the 96 hour test period. In both mice and men the amount of the drug entering the red blood cells was approximately 7 per cent of the total drug found in the blood at any given time.

A comparison was made of the effectiveness of 3-4, Dimethyl-5-sulfanilamido-isoxazole with that of sulfadiazine in controlling experimental infections produced in mice by Strep. hemolyticus, D. pneumoniae (Type I) Kl. pneumoniae (Type A) and H. influenzae (Type b). In mice fed with this compound or sulfadiazine for a total of 7 days in amounts adequate to produce similar concentrations of these drugs in the blood of the animals, and infected with similar doses of Strep. hemolyticus (C-203) by the intraperitoneal route after 48 hours of the drug feeding, the protective action of this drug was comparable to that of sulfadiazine, if not somewhat better. The same was true when D. pneumoniae (SVI) was the infecting organism. When Kl. pneumoniae (Type A) was employed to produce the

* These investigations were supported by grants from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories, Inc., Parke, Davis and Company, and the Upjohn Company.

infections, sulfadiazine was more effective. In infections produced in mice by the inoculation of *H. influenzae* (Type B) neither drug was effective in the doses employed.

CONCLUSIONS

1. The experimental acute toxicity of 3-4, Dimethyl-5-sulfanilamido-isoxazole is low.
2. The drug appears to be only partially absorbed from the gastro-intestinal tract.
3. The rate of acetylation of this compound in the tissues and body fluids seem to be low.
4. In the blood, very little of the drug enters the blood cells as approximately 93% of the compound is found in the plasma.
5. This sulfonamide derivative seems to be comparable to sulfadiazine in its ability to protect mice against Beta hemolytic streptococcal and pneumococcal infections. It is not as effective as sulfadiazine in experimental infections in mice produced by *Kl. pneumoniae*. In the experiments reported in this abstract neither compound was protective against experimental infections produced by *H. influenzae* Type B.
6. Clinical trials with 3-4, Dimethyl-5-sulfanilamido-isoxazole would appear justifiable.

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Dr. E. K. Marshall, Jr.: When Dr. Schnitzer's paper came out a year or so ago this drug interested me very much. I think one of the most interesting things about it (Dr. Long didn't mention it) is the fact that it and its acetyl derivatives are about as soluble as sulfanilamide. It was predicted that this compound would not cause renal concretions. We have made a few studies on it. It is distributed in the dog and in the human being only in extracellular water. In the dog its apparent volume of distribution is about 25 to 30 per cent of the body weight, and in man about 20 to 25 per cent, which corresponds roughly to the extracellular water. The importance of this observation is that with a drug present only in extracellular water one would not expect it to exhibit certain toxic properties which would be exhibited by a drug in intracellular fluids. Sulfanilamide and sulfapyridine, the first sulfonamides to be used clinically, are distributed in 80 to

85 per cent of the body weight and have certain toxic characteristics which are not shared by sulfadiazine, which is distributed in 45 to 55 per cent of body weight. I would predict that this new drug will show far less toxicity than the other sulfonamides. Another advantage of this drug being distributed only in extracellular water is that with the same dose of this drug one would obtain three times as high a plasma level as you would with sulfanilamide or sulfapyridine.

Dr. Arnold R. Rich: I would like to ask Dr. Marshall, when he says toxicity does he mean hypersensitivity also? Because there, of course, the drug combines with the plasma protein in most instances.

Dr. Marshall: No, I don't think it would have any effect on that.

*In Vitro Studies of Polymyxin.** DRS. ELEANOR A. BLISS, CAROLINE A. CHANDLER and EMANUEL B. SCHOENBACH (*Department of Preventive Medicine, The Johns Hopkins University School of Medicine*).

Polymyxin is a new antibiotic agent the activity of which is restricted to gram-negative bacteria. Benedict and Langlykee (1) were the first to report that extracts of *B. polymyxa* inhibited bacterial growth. Quite independently Stansly and his associates (2, 3, 4) described the active principle and named it "polymyxin". Meanwhile Brownlee et al (5, 6) reported on "aerosporin" which appears to be similar but not identical with polymyxin.

The observations of other investigators regarding certain of the physicochemical properties, as well as the antibacterial action of polymyxin have been confirmed. The agent, *in vitro*, is most effective in an acid environment. It is quite stable to heat and loses but little activity in the presence of serum. The combination of heat and serum, however, is deleterious. Most strains of *E. coli*, *Aerobacter* and Friedlander's bacillus are susceptible to 0.3 microgram of polymyxin per cc., when an inoculum of about 200,000 bacilli is used. *H. influenzae* is equally susceptible. *Pseudomonas aeruginosa* required 2.5 µg./cc., while strains of *Proteus* and meningococci appear to be resistant *in vitro*. Attempts to produce resistance in sensitive strains have been unsuccessful. End points in titrations are clear cut and further incubation does not cause them to shift. This observation and studies of growth curves show that the drug is bactericidal in concentrations above 0.2 µg./cc. However, the addition of polymyxin at intervals after growth has started fails to result in sterilization of the cultures. This may be due to the fact that the size of the inoculum is extremely important. Large inocula of *E. coli* grow out in relatively high concentrations of the agent. Such cultures, freed of bacteria, are found to have lost much of their polymyxin activity, either because of destruction or adsorption of the agent. Aqueous emulsions of soy bean lecithin also interfere with the action of polymyxin.

* These investigations were supported by grants from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories, Inc., Parke, Davis and Company, and the Upjohn Company.

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*The Experimental Toxicology, Pharmacology, and Effectiveness of Polymyxin.** DRS. MORTON S. BRYER, EMANUEL B. SCHOENBACH, ELEANOR A. BLISS and C. EARL OTT (Department of Preventive Medicine, The Johns Hopkins University School of Medicine).

The acute toxicity of "polymyxin" was determined in mice by single subcutaneous injection and the L.D. 50 was 250-300 mg/kilo. Dogs survived single, rapid intravenous injections of 10 and 15 mg/kilo, while 25 mg/kilo resulted in convulsions, apnea, and death. Continuous intravenous administration at the rate of 0.25 mg/kilo per minute resulted in paralysis, apnea, and death when 35 mg/kilo had been given. Dogs tolerated 5 and 10 mg/kilo intramuscularly twice daily for 7 days. Intrathecal injection of 1 and 5 mg. was associated with no untoward reaction while 10 mg. produced transient paresis.

Ninety minutes after single intramuscular doses of 5 and 10 mg/kilo in dogs' serum levels of 2.5 and 5.0 gamma/cc. were obtained. Three and a half hours after these injections serum contained 2.5 and 1.25 gamma/cc. respectively. Levels obtained were approximately four times higher when dogs had been maintained on these doses twice daily for 7 days. Despite persistent high serum levels no polymyxin was detected in the spinal fluid. Intrathecal administration of 1, 5, and 10 mgs. resulted in spinal fluid levels of 10-500 gamma/cc. which fell to 0.3-20 gamma/cc. in 2 to 4 hours. Serum levels of 0.6-1.25 gamma/cc. were obtained following intrathecal doses of 5 and 10 mgs.

Single subcutaneous injection of 1 mg/kilo protected the majority of mice infected intraperitoneally with 1000 L.D.'s of *Kl. pneumoniae* A or *H. influenzae* B. A single initial dose was more effective than the same amount of "polymyxin" given in divided doses. Delay in treatment resulted in a reduction in the number of survivors. Polymyxin was more than five times as effective as streptomycin by weight.

* These investigations were supported by grants from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories, Inc., Parke, Davis and Company, and the Upjohn Company.

*The Clinical Use of Polymyxin.** DRs. EMANUEL B. SCHOENBACH, MORTON S. BRYER, ELEANOR A. BLISS and PERRIN H. LONG (*Department of Preventive Medicine, The Johns Hopkins University School of Medicine*).

The clinical trial of "polymyxin" for the treatment of patients ill with infections caused by gram-negative bacteria appeared desirable in light of the wide differential between toxicity and therapeutic activity observed in experimental animals. The inability to demonstrate the development of resistance to this antibiotic was a distinct advantage when compared to previous experience with streptomycin.

The dosage employed in these early cases varied from 3.0 to 5.0 milligrams per kilogram of body weight daily. The total daily amount was divided into eight equal parts and administered intramuscularly every 3 hours. The drug was dissolved in a phosphate buffer pH 7.6 and 40-50 milligrams in one cubic centimeter caused no local irritation or discomfort. Blood, urine and spinal fluid samples were obtained at various intervals for bioassay of polymyxin levels. Blood levels varied from 1.3 to 20 gamma per cubic milliliter on this dosage regime. The higher levels were usually not attained until the seventh to tenth day of treatment. None of the polymyxin was detected in the spinal fluid following intramuscular injection, but on one occasion a level of 5 gamma per cubic milliliter was noted 12 hours after the intrathecal administration of 2.0 milligrams of drug. Urinary excretion of polymyxin was low in the first 12 hour period but increased exponentially as therapy was continued, so that approximately 60 per cent of the total drug was detected 96 hours after administration had been begun. The concentration of polymyxin in the urine varied from 0.25 gamma to 160.0 gamma per cubic milliliter. Levels above 1.0 gamma were not attained until approximately 24 hours after therapy had been instituted.

Five patients ill with infections due to *Ps. aeruginosa*, *Br. abortus* and *Kl. pneumoniae* have been treated for periods of eleven to twenty-nine days. The drug was well tolerated. Among these severely ill patients, two showed transitory albuminuria and one a diffuse macular eruption. These were not attributed to polymyxin. On two occasions a transitory vasomotor reaction 10-20 minutes after an injection was noted. One patient developed febrile response on the 17th day of treatment. His temperature fell to normal when treatment was discontinued. The clinical course of these patients treated with polymyxin indicate that the antibiotic was beneficial. The results confirm previous observations on the chemotherapy of experimental infections in mice.

Dr. Harold J. White: I would like to say that the results obtained in our laboratory are in complete agreement with the findings that Dr. Bliss and Dr. Bryer have reported here tonight. We are convinced that: (1) polymyxin is practically

* These investigations were supported by grants from Abbott Laboratories, Eli Lilly and Company, Lederle Laboratories, Inc., Parke, Davis and Company, and the Upjohn Company.

specific for gram-negative bacteria; (2) it is bactericidal, and rapidly so; (3) in experimental infections, the total daily dose is more effective when given all at once than when divided into frequent doses; (4) with polymyxin, one would not expect to encounter bacterial resistance as a serious problem. There seems to be no discrepancy between our results and those just reported.

Dr. Rich: I would like to ask Dr. Schoenbach if bacillus pertussis was recovered from either of those cases which he mentioned.

Dr. Horace L. Hodes: Pertussis organisms were not recovered from either of the patients described by Doctor Schoenbach. We not infrequently fail to recover pertussis bacilli from young infants with typical whooping cough. I do not believe that there is any doubt about the diagnosis. We cannot be sure that the polymyxin was of any benefit. Our impression is that the course of the disease in the younger infant was greatly shortened. About the older child, we can draw no conclusion whatever. We have found in the laboratory that between 0.5 and 2.0 micrograms of polymyxin per cc. of media inhibits the growth of *Hemophilus pertussis*. Preliminary experiments indicate that polymyxin has a definite protective action against this organism in mice. Regarding the child with the *Pseudomonas aeruginosa* (pyocyaneus) infection, I do not believe that there is any doubt but that polymyxin was of great benefit. I am quite convinced that the child would not have survived unless he had been treated with this drug.

Dr. Marshall: I would like to ask if any changes were found in the urine except in one case, the first one. I realize that we are working with a very impure mixture. Until this substance is obtained pure we must always be suspicious as to whether the pure antibiotic causes the reaction or whether it is due to the impurities present. Were there any changes in the dogs' urine, Dr. Bryer?

Dr. Morton S. Bryer: I didn't examine the urine.

Dr. Hodes: In the case of that first child, there were changes in his urine when he came into the hospital.

Dr. Emanuel B. Schoenbach: All these patients have been acutely and seriously ill. This child had 3-plus albuminuria, 104° fever and pyuria, which disappeared while on therapy. It is a question whether polymyxin caused the albuminuria or whether it was just due to the febrile response.

Dr. Perrin H. Long: Dr. Schoenbach said that the dosage of polymyxin was arbitrary. It was and was put upon Dr. Schoenbach and Dr. Bryer by myself because, in introducing a new antibiotic, I wanted to err as much as possible on the side of safety to the patient. In the experimental animals, it is interesting that this antibiotic has shown greater acute toxicity than any other antibiotic with which we have worked. Small and divided doses are safer to use in the early part

of such studies until we know more about its toxicity in human beings. As a result of our experience to date, I think that in the near future we will give the antibiotic less frequently, and will give half the daily total as the initial dose, and then a quarter of the total daily dose 8 and 16 hours later, and then a third of the daily dose at 8 hourly intervals on subsequent days. We will see whether this dosage schedule is more effective in bringing the disease under control early in the course of treatment. It is difficult, when you are fishing around in the dark, to hit upon exactly the right dosage schedule, and, as I said, you always want to err on the side of safety in working with any new antibiotic.

Dr. Rich: Were there any pathological studies made with animals?

Dr. Long: No, no pathological studies were made on the animals that died of acute toxicity. There was no real evidence of chronic toxicity as far as we went. There were no pathological studies made on the mice which died, or on the dogs. We would like to have some one make the studies, especially on those animals that died acutely in convulsions. We would like to know what the drug does to the central nervous system. The organs are preserved in formalin.

Dr. A. Murray Fisher: I was interested in Dr. Bliss' report of the apparent development of resistance of the organisms during their growth with low concentrations of polymyxin. I wonder if she tried spinning the cultures down to separate them from the media, regrowing them, and retesting them with polymyxin.

Dr. Eleanor A. Bliss: What we did was to take the bacteria which had grown in the presence of polymyxin, large inocula of *E. coli* for instance, which had grown out, and used that, diluting it down to usual size, 200,000 per cc. We found it was not resistant. When we centrifuged, we were trying to see whether the polymyxin that was left in the tube was as strong as what was there in the beginning. The cultures have all been grown between testing for resistance and at the time they were in the tube.

Dr. Fisher: I mean, when you spun the cultures down, did you separate the bacteria from the media and test them for resistance?

Dr. Bliss: No, we did not spin the cultures down and test them for resistance. We used them as broth cultures and just diluted them.

Dr. Long: Another thing which Dr. Schoenbach or Dr. Bryer may have spoken about tonight was rather puzzling. As you noted on one of those charts, meningococci on plate cultures were quite resistant to polymyxin, even to 20 to 30 micrograms of the antibiotic per cubic centimeter. However, when the antibiotic was injected in mice infected by using mucin and meningococci we found that polymyxin in a single dose cured 100 per cent of the mice. Now, there is a difference between in vitro and in vivo findings. We are still working to try to explain the difference in reaction.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Emotional Maturity. By LEON J. SAUL. 338 pp. \$5.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*

In this book by Dr. Saul, Associate Professor of Psychiatry at the Temple University School of Medicine, the focus of interest throughout is clinical and therapeutic. As indicated by the title, Dr. Saul employs a framework of reference which includes attention not only to dynamic pathological patterns but also to the biological process of emotional growth and to faults in this process as a source of adult maladjustment.

The material of the book is presented in four parts. To the present reviewer, the discussion in Part I, the Achievement of Maturity, and Part II, Emotional Forces in the Development of the Personality, seemed most valuable, and alone would have justified the publication of this book. Here the author formulates his concept of emotional maturity and of the maturation process. Some of the central features which he depicts are the shifts which the individual is required to make in his functioning in relationships with others in the course of his maturation: the shift from the childhood pattern of dependence, freedom from responsibilities and being protected to the adult capacity for carrying responsibility, being depended on and providing protection; the shift from primarily *getting* in relationships with others to enjoying *giving*; and the shift from egotism, competitiveness and inferiority feelings to a kindly and cooperative adjustment to others, with enjoyment of one's own productive activity. The persistence of certain childhood patterns into chronological adulthood is described as leaving the individual with vulnerable emotional points—the "emotional Achilles heel." The presence of these vulnerable emotional points makes the individual sensitive to specific situational stresses while leaving him resistant to other severe stresses which would be disturbing to another individual with another set of vulnerable points. The author makes the point that reservoirs of childhood needs remain even in adults who have attained a high degree of emotional maturity and that these needs are filled in play and recreational outlets.

Part III, The Nature of Neurosis, is almost a book within a book. It is based on the author's war-time clinical experience with navy psychiatric casualties and is rich in clinical material. This case material is aptly selected and is presented with clarity and enviable brevity. It serves as a basis for illustrating the concept of specific emotional vulnerability and for a consideration of the meaning of various emotional responses such as fear, hostility, flight, fight, etc. To Dr. Saul the term neurosis implies "a failure of adaptation and a disturbance of the patient's emotional development." The nature of neurosis is "reaction to stress upon vulnerable

points and fight-flight reactions along the lines of childhood patterns." With regard to therapy the author states that, in contrast to the results produced by rest and temporary relief from duty, "circumscribed but carefully focused psychotherapy, aimed at the specific source of the patient's reactions, produced marked results after only one or two interviews."

Part IV, *The Dynamics of Personality*, reads rather like lectures to medical students. It is a brief section and neither adds nor detracts from the book as a whole.

There is a reference list of 112 titles, and an index.

B. B.

Gynecological and Obstetrical Urology. 2nd Edition. By HOUSTON S. EVERETT. Illus. 539 pp. \$6.00. *The Williams & Wilkins Company, Baltimore, Maryland*, 1947.

Dr. Everett has made a number of timely changes and additions in the second addition of his textbook. A section on indirect, or "water", cystoscopy has been added since most urologists employ this method rather than the direct, or Kelly method. Indications for use of the two methods are given, and the common indirect cystoscopic instruments are illustrated and described. Other additions to the text include a discussion of the use of penicillin in urinary tract infections and a description of the Aldridge operation for urinary incontinence, with illustrations demonstrating the operative technique. The chapter on "Neoplastic Disease of the Bladder" has been completely rewritten, stress being laid on the importance of *infiltration* rather than the gross or microscopic appearance of the tumor alone. Dr. Everett emphasizes that all these factors, together with the characteristics of the tumor on pelvic examination, must be considered before the type of treatment is determined in any particular case. This textbook has always been a very complete one, and the present edition brings it up to date. The style is clear and the viewpoint sound. Dr. Everett is to be congratulated on producing an excellent book. In the writer's opinion, it is the best and most up to date work of its kind in the literature of today.

C. B. B.

Jaundice, Its Pathogenesis and Differential Diagnosis. By ELI RODIN MOVITT. Illus. 261 pp. \$6.50. *Oxford University Press, New York, New York*, 1947.

The difficulties which the clinician meets in the differential diagnosis of jaundice are reflected in the frequency with which the subject is reviewed in the medical literature. Movitt has attempted "to aid the practicing physician" in this task. He reviews in orderly fashion the anatomy and physiology of the liver, the pathogenesis of jaundice, diagnostic procedures, and the differential diagnosis of the individual syndromes associated with jaundice. Interspersed throughout the text are tables summarizing the points which Movitt believes require emphasis.

The arrangement of subjects is clear and logical. Certain features, however, tend to limit the utility of this book. The textual material is presented didacti-

cally and usually without reference to those authors upon whose shoulders Movitt seems to have leaned heavily. The bibliography at the end of each chapter is not helpful, since it is not possible to tell to which subject any given article refers.

There are a number of textual and bibliographic inaccuracies as well. For example, prothrombin is usually believed to be associated with the globulin rather than the albumin fraction of human plasma. The reviewer, too, might take issue with some of the author's concepts, but, in a controversial field, differences of opinion are surely healthy. Thus, one might question the separation of acute infectious hepatitis and catarrhal jaundice. Again, in our hands, the determination of serum alkaline phosphatase has been one of the most satisfying of differential diagnostic procedures. The format and printing of this volume are pleasing and attractive. None of the deficiencies of the book are beyond repair in a future edition.

O. D. R.

BOOKS RECEIVED FOR REVIEW

- Catalogue of Medical Films.* Compiled by THE ROYAL SOCIETY OF MEDICINE AND THE SCIENTIFIC FILM ASSOCIATION. 127 pp. 7s.6d. *Aslib, London, W.C.1*, 1948.
- Epithelia of Woman's Reproductive Organs, The.* By GEORGE N. PAPANICOLAOU, HERBERT F. TRAUT, AND ANDREW A. MARCHETTI. Illus. 53 pp. \$10.00. *The Commonwealth Fund, New York 22, New York*, 1948.
- Headache, and other Head Pain.* By HAROLD G. WOLFF. Illus. 642 pp. \$12.00. *Oxford University Press, New York, New York*, 1948.
- Integrative Action of the Nervous System, The.* 2nd edition. By SIR CHARLES SHERRINGTON. Illus. 433 pp. \$6.00. *Yale University Press, New Haven, Connecticut*, 1948.
- Introduction to Human Physiology.* By WILLIAM D. ZOETHOUT. Illus. 424 pp. \$4.00. *C. V. Mosby Company, St. Louis, Missouri*, 1948.
- Pathology of Tumours.* By RUPERT A. WILLIS. Illus. 992 pp. \$20.00. *C. V. Mosby Company, St. Louis, Missouri*, 1948.
- Psychobiology and Psychiatry.* 2nd edition. By WENDELL MUNCIE. Illus. 620 pp. \$9.00. *C. V. Mosby Company, St. Louis, Missouri*, 1948.
- Symposium on Medicolegal Problems.* Edited by SAMUEL A. LEVINSON. 255 pp. \$5.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania*, 1948.
- Textbook of Gynecology.* 3rd edition. By EMIL NOVAK. Illus. 742 pp. \$8.00. *The Williams & Wilkins Company, Baltimore, Maryland*, 1948.
- Topics in Physical Chemistry.* By W. MANSFIELD CLARK. Illus. 738 pp. \$10.00. *The Williams & Wilkins Company, Baltimore, Maryland*, 1948.
- Treatment by Diet.* 5th edition. By CLIFFORD J. BARBORKA. Illus. 784 pp. \$10.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania*, 1948.

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